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(アイウエオ順)

論　説

Studies on the Nucleus of the Spore in *Streptomyces*. Part-II. Observations on the Nuclear Substances of the Spore in *Streptomyces* which belong to B and C groups.

by Shukuo KINOSHITA* and Shiro ITAGAKI*

木下祝郎*・板垣史郎:* 放線菌胞子の核に関する研究. 第2報 B および C 群に属する放線菌胞子の核物質の観察

Received September 27, 1958

The nucleus of the spore in nine strains of *Streptomyces* which belong to A group was observed in the previous report[1].

In this report, the nuclear substances of the spore in thirteen strains which belong to B group and eithteen strains which belong to C group were observed. While the strains of B group produce the soluble pigment on the organic media, the strains of C group do not. This difference has been cleared up in Bergey's manual[2].

The methods of incubation and experiments were described in Part-I previously.

Morphological observations of spores were done with the aid of the electron-microscope to all strains tested.

Experiments and results

1. Observations on the nuclear substance of the spores in various species of *Streptomyces* which belong to B group.

1) *S. roseochromogenus* 0-36-738

2) *S. roseochromogenus* B-2

These strains did not show the typical nuclear figures. Both the polar parts of the spore were stained deeply as shown in Fig. 1. This observation was similar to the pair of chromosomes in secondary hyphae which were descrived by Klieneberger-Nobel[3]. But, it is rather unreasonable that the results of the present observation were analogous to Klieneberger-Nobel's pair of chromosomes at the stage of nucleus-forming because the present materials sporulated completely on Bennet's agar slant.

3) *S. cinnamonensis* 154–T3

Always uninucleate, but rod- or dumbbell-shaped nucleus or two nuclei were observed rarely.

4) *S. flavochromogenes* T349-15

Always uninucleate, but large long ellipsoidal nucleus was observed.

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5) *S. flavochromogenes* H-3206

Always uninucleate. This strain resembles with T349-15 strain, but thick rod-shaped nucleus was observed.

6) *S. antibioticus* U-1091

Details were obscure because the clear preparations were not obtained by usual method. This strain may be uninucleate.

7) *S. viridochromogenes* CBS (IFO-3113)

Most of the spores contained spherical nuclei. But there were abnormally large spores which contained diffuse or very large nuclei.

8) *S. pheochromogenus* 253

There was spherical or ellipsoidal nucleus at the center of long ellipsoidal or cylindrical spore. Always uninucleate.

9) *S. pheochromogenus* W-41

In most of the cases of this strain the spore was uninucleate, but dumbbell shaped nucleus or two nuclei were rarely observed in the spore.

There were so many spores which had specially large nucleus. It seems that specially large nucleus was correlated to the maturation of spores because these spores were combined with usual spores in the same sporechain. These large nuclei were easily stainable with Giemsa and had various shapes. Specially large nucleus appeared only in the spore of *S. pheochromogenes* W-41 in thirteen tested strains which belong to B group.

Table 1. Distribution of the type of nuclei in *S. pheochromogenus* W 41.

type of nucleus	Distribution %
	97
 	2.5
	0.5

Distributions of shapes and numbers of nuclei were shown in Table 1.

10) *S. pheochromogenus* S-66-B

Always uninucleate. There was a spherical or ellipsoidal nucleus in a spore. The nucleus was shown larger in a microphotograph due to the halation and the nucleus was easily stainable with Giemsa.

11) *S. aureus* ATCC-3309

Always uninucleate.

12) *S. erythrochromogenes* W-115C

Most of the spores were uninucleate, but dumbbell-shaped nucleus and two nuclei were observed in about eight percent cases. In rare cases comma-type nuclei were observed.

Table. 2. Distribution of the type of nuclei in *S. erythrochromogenes* W-115C

type of nucleus	Distribution %
	87
	4
	4
	5

Distributions were shown in Table 2..

13) *S. lavendulae* ATCC-8664

Table 3. Distribution of the type of nuclei in *S. lavendulae* ATCC-8664.

type of nucleus	Distribution %
	62
	18
	12
	5
	3

Rod- or dumbbell-shaped nucleus was observed in relatively many cases.

Trinucleate spores were observed in rare cases. Distributions were shown in Table 3.

The thread-like structure which jointed with separated two nuclei was observed as shown in *S. griseoflavus* #305.

Distribution of the number and type of nuclei in the spores of thirteen strains of *Streptomyces* which belong to B group are shown in Table 4. When counted, rod-shaped nucleus was counted as uninucleate and dumbbell shape nuclei counted as two nuclei.

Table 4. Distribution of the number and type of nuclei in the spores of *Streptomyces* which belong to B group.

Strains	number %			type %				
	1	2	3	—	—	—	—	other
S. roseochromogenus O-36-738	obscure							
S. roseochromogenus B-2	obscure							
S. cinnamonensis 154-T3	100	rare	—	100	rare	rare	rare	—
S. flavochromogenes T349-15	100	—	—	100	rare	—	—	—
S. flavochromogenes H-3206	100	—	—	100	rare	—	—	—
S. antibioticus U-1091	100	—	—	100	—	—	—	—
S. viridochromogenes CBS (IFO 3113)	100	—	—	100	—	—	—	diffuse nucleus
S. pheochromogenus 253	100	—	—	100	—	—		
S. pheochromogenus W-41	97	3	—	97	—	2.5	0.5	—
S. pheochromogenus S-66 B	100	—	—	100	—	—	—	—
S. aureus ATCC-3309	100	—	—	100	—	—	—	—
S. erythrochromogenes W-115C	92	8	—	87	—	4	4	5
S. lavendulae ATCC-8664	83	17	rare	62	18	12	5	3

2. Observations on the nuclear substance of the spores in various species of *Streptomyces* which belong to C group.

1) *S. microflavus* 13-A

Not only the forms of spores of this strain were irregular morphologically but also the shape of nucleus is variable. When a spore has one nucleus, for example, the shape of the nucleus varies from spherical to rod-shaped. Three nuclei were

rarely observed in a spore.

These distributions were shown in Table 5.

Table 5. Distribution of the type of nuclei in *S. microflavus* 13-A

type of nucleus	Distribution %
	82
	10
	2.5
	5.5

2) *S. griseus* #1

The form of spores and the shape of nucleus were relatively uniform. Always uninucleate, but dumbbell-shaped unclei or two nuclei in a spore were rarely observed.

3) *S. griseus* SX_2-O-11

4) *S. griseus* H-12

5) *S. griseus* TS-601

6) *S. griseus* SN-14

These strains were always uninucleate, but two nuclei were rarely observed in *S. griseus* SN-14.

7) *S. griseus* MC-33

Always uninucleate, but rod- or dumbbell-shaped nucleus or two nuclei were rarely observed.

Twenty minutes hydrolysis at 50° gave better results than ten minutes hydrolysis at same temperature.

8) *S. olivaceus* NRRL-B-1125

Table 6. Distribution of the type of nuclei in *S. olivaceus* NRRL-B-1125.

type of nucleus	Distribution %
	78
	7
	4
	8
—	3

There were various shapes of nuclei. Of course, most of the spores were uninucleate, but these nuclei were varied from spherical to rod- shaped. Two nuclei and three nuclei were not so rarely in a spore.

These distributions were shown in Table 6.

9) *S. olivaceus* CR-74

Most of the spores were uninucleate, but there were spores which have rod-shaped or, comma-shaped nucleus or two nuclei. The none-nucleate spores were rarely observed.

10) *S. olivaceus* PD-60

There were rod-shaped nucleus or two nuclei in some cases.

These distributions were shown in Table 7.

Table 7. Distribution of the type of nuclei in *S. olivaceus* PD-60.

type of nucleus	Distribution %
	96
	2.5
	1.5

Two kinds of spores were observed. The larger one had a large nucleus and the smaller one had a small nucleus.

11) *S. olivaceus* G

Uninucleate, but there was rod-shaped nucleus rarely.

Table 8. Distribution of the type of nuclei in *S. sahachiroi*.

type of nucleus	Distribution %
	95
	5
	rare

12) *S. olivaceus* GSM^r_{5000}

This strain was obtained as a resistant strain for streptomycin 5000γ/ml. The parent strain is *S. olivaceus* G.

Both the polar parts of spore were stained with Giemsa as in the case *S. roseochromogenes* and ring nuclei were observed.

The detail of the structure is not yet clarified.

13) *S. sahachiroi*

There was one nucleus in an oval spore which is very uniform in size and form. But dumbbell-shaped nucleus was observed at the rate of five percent. Two nuclei were rarely observed.

These distributions were shown in Table 8.

Table 9. Distributions of the number and type of nuclei in the spores of *Streptomyces* which belong to C group.

Strains	number %			type %				
	1	2	3	——	——	——	——	others
S. microflavus 13-A	92	8	——	82	10	2.5	5.5	——
S. griseus #1	100	rare	——	100	——	rare	rare	——
〃 SX_2-O-11	100	——	——	100	——	——	——	——
〃 H-12	100	——	——	100	——	——	——	——
〃 TS-601	100	——	——	100	——	——	——	——
〃 SN-14	100	——	——	100	——	——	——	——
〃 MC-33	100	rare	——	100	rare	rare	rare	——
S. olivaceus NRRL-B-1125	85	12	3	78	7	4	8	3
〃 CR-74	98.5	1.5	——	96	2.5	——	1.5	comma-type
〃 PD-60	100	——	——	100	rare	——	——	——
〃 G	100	——	——	100	rare		——	——
〃 GSM^r_{5000}	obscure			obscure				
S. sahachiroi	95	5	——	95	——	5	rare	——
S. halstedii CBS (IFO-3199)	100	——	——	100	——	——	——	——
S. lipmarii 3331	100	——	——	100	——		——	——
S. fradiae 117	100	——	——	100	——	——	——	——
〃 3535	obscure	——	——	obscure	many	many	rare	——
〃 (Okami)	100	——	——	100		——	——	——

14) *S. halstedii* CBS (IFO-3199)

Uninucleate, but the details were obscure. There were some none-nucleate spores.

15) *S. lipmanii* 3331

Always uninucleate, but the shape of nucleus was various from spherical to rod-like.

16) *S. fradiae* 117

Always uninucleate, and the shape of nucleus was very uniform.

17) *S. fradiae* 3535

Uninucleate, but rod- or dumbbell shaped nucleus were often observed. Two nuclei were also observed rarely.

These distributions could not shown as a table.

18) *S. fradiae* (Okami)

Always uninucleate. Spores were slightly irregular in form and size.

Distributions of the number and type of nuclei in the spores of eighteen strains of *Streptomyces* which belong to C group were shown in Table 9.

Discussion

1. On the nucleus of spore in *Streptomyces* which belong to B group.

Seven strains out of the thirteen were always uninucleate, but two nuclei were clearly observed in *S. lavendulae* ATCC-8664 (17 %), *S. erythrochromogenes* W-115C (8 %), *S. pheochromogenus* W-41 (3 %) and *S. cinnamonensis* 154-T3 (rarely). Tri-nucleate spores were observed in *S. lavendulae* ATCC-8664 very rarely.

Rod- or, dumbbell-shaped nuclei and comma-type nuclei were observed. Comma-type nucleus could not observed in observed nine strains of *Streptomyces* which belong to A group.

Nuclei of *S. roseochromogenus* O-36-738 and B-2 were not stained by usual technique. Both the polar parts of the spore were stained deeply.

2. On the nucleus of spore in *Streptomyces* which belong to C group.

Twelve strains out of the eighteen were always uninucleate, but binucleate spores were clearly observed in *S. olivaceus* NRRL-B-1125 (12 %), *S. microflavus* 13-A (8 %), *S. sahachiroi* (5 %), *S. olivaceus* CR-74 (rarely), *S. griseus* #1 (rarely) and *S. griseus* MC-33 (rarely). Trinucleate spores were observed in *S. olivaceus* NRRL-B-1125 only. The ring nucleus was observed in *S. olivaceus* GSM^{r}_{5000}. This strain is a mutant of *S. olivaceus* G and it is resistant against streptomycin 5000γ/ml.. It seems that there are some relationships between the shape of nucleus and resistant character.

We observed that there were some none-nucleate spores in *S. olivaceus* CR-74.

Summary

The number and shape of nuclei in the spores of *Streptomyces* which belong to B and C groups were observed. The results were as follows:

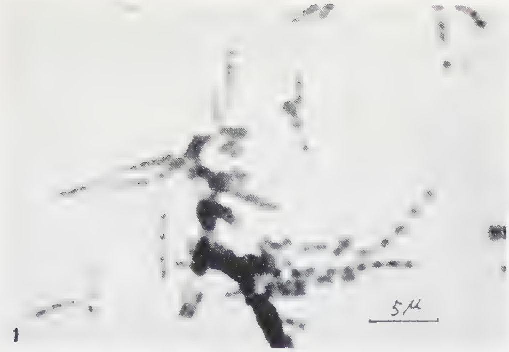

Fig. 1

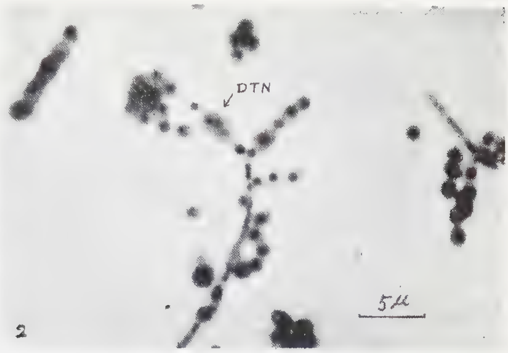

Fig. 2

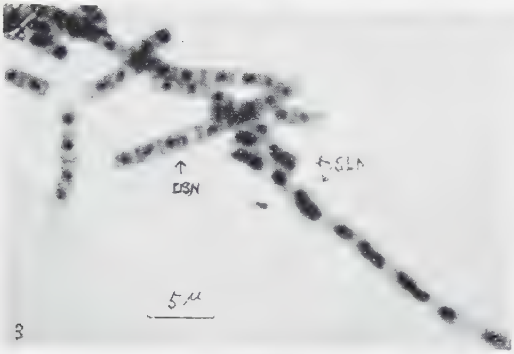

Fig. 3

Fig. 4

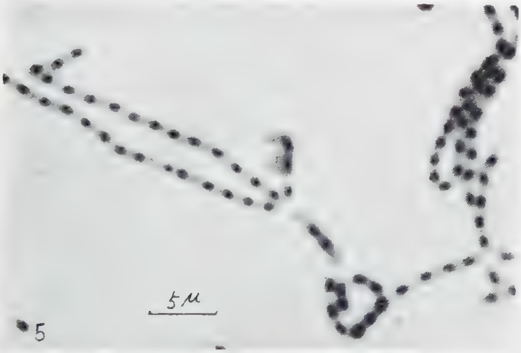

Fig. 5

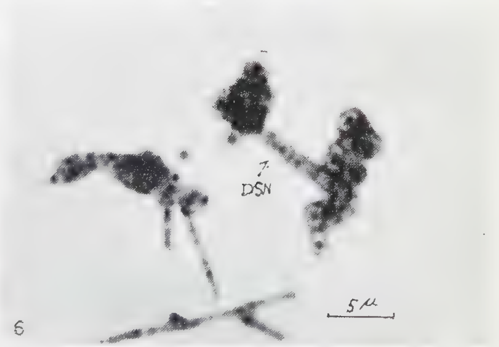

Fig. 6

Fig. 7

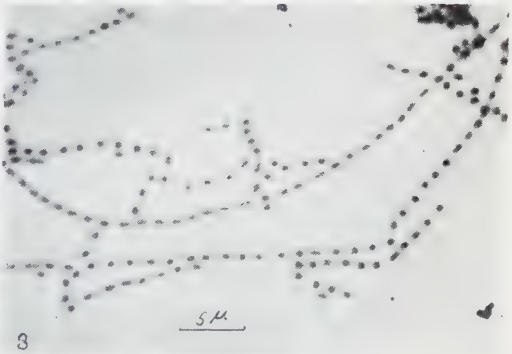

Fig. 8

Fig. 1. *S. roseochromogenus* 0-36-738. Both the polar parts of the spore were deeply stained with Giemsa. The nucleus does not show typical form. Fig. 2. *S. viridochromogenes* CBS (IFO-3113). Abnormally large spores which have diffuse or large nucleus are shown. Fig. 3. *S. pheochromogenus* W-41. Specially large nuclei are shown. Fig. 4. *S. pheochromogenus* S-66-B. Nuclei are uniform in shape and size. Fig. 5. *S. aureus* ATCC-3309. Uninucleate. Fig. 6. *S. erythrochromogenes* W-115 C. Dumbbell-shaped nucleus is clearly shown. Fig. 7. *S. lavendulae* ATCC-8664. Various types of nuclei are shown. Fig. 8. *S. griseus* #1. Uninucleate.

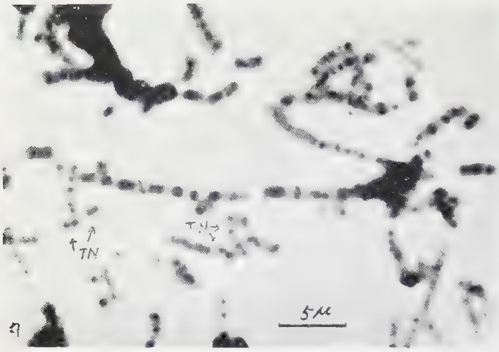

Fig. 9

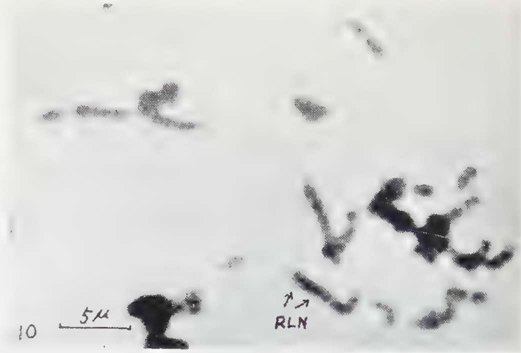

Fig. 10

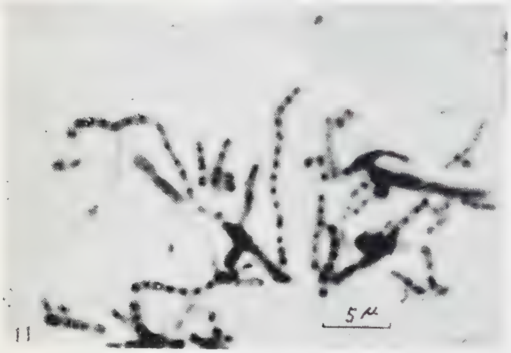

Fig. 11

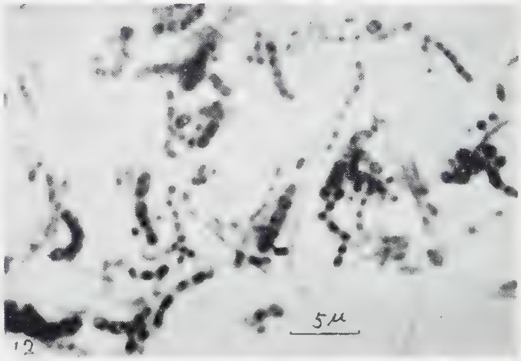

Fig. 12

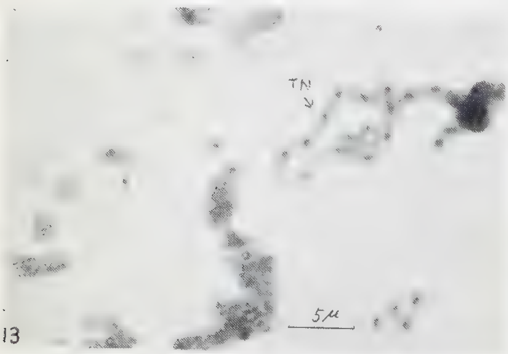

Fig. 13

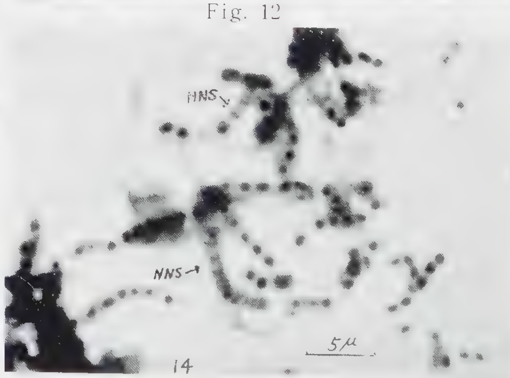

Fig. 14

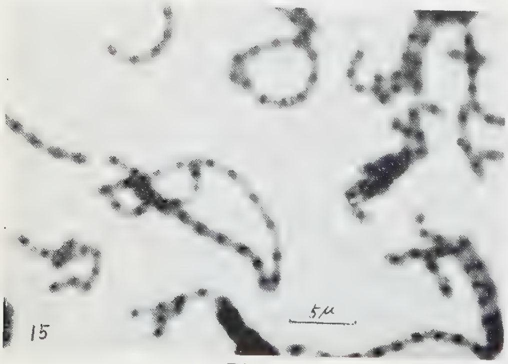

Fig. 15

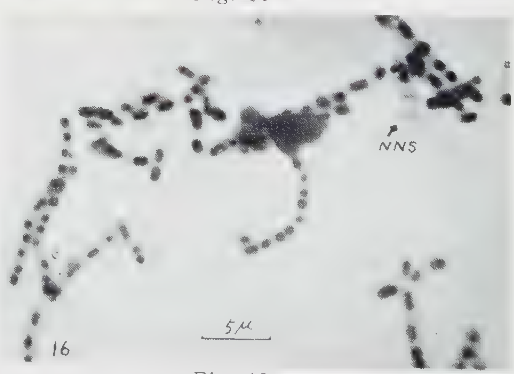

Fig. 16

Fig. 9. *S. olivaceus* NRRL-B-1125. Various types of nuclei are shown. Fig. 10. *S. olivaceus* PD-60. Two sizes of spores are observed. The larger one has larger nucleus than the smaller. Fig. 11. *S. olivaceus* G. Fig. 12. S. *olivaceus* GSM^{r}_{5000}. Ring nuclei are observed. The shape of nucleus of this strain differs from its parent strain, *S. olivaceus* G. Fig. 13. *S. sahachiroi*. Fig. 14. *S. halstedii* CBS (IFO-3199). None-nucleate spores are shown here and there. Fig. 15. *S. fradiae* 117. Fig. 16. *S. fradiae* 3535.

1. On B group.

1) Seven strains out of thirteen most spores were uninucleate. The shape of nucleus seems spherical or ellipsoidal.

2) In four strains out of thirteen, *S. lavendulae* ATCC-8664 (17 %), *S. erythrochromogenes* W-115C (8 %), *S. pheochromogenus* W-41 (3 %) and *S. cinnamonensis* 154-T3 (rarely), two nuclei were observed.

3) Trinucleate spores were rarely observed only in *S. lavendulae* ATCC-8664.

4) Specially large nucleus was observed in *S. pheochromogenus* W-41.

5) Nucleus could not be observed in two strains of *S. roseochromogenus*.

2. On C group.

1) On twelve strains out of eighteen most spores were uninucleate. The shape of nucleus seems spherical or ellipsoidal.

2) In six strains out of eighteen, that is, *S. olivaceus* NRRL-B-1125 (12 %), *S. microflavus* 13-A (8 %), *S. sahachiroi* (5 %), *S. olivaceus* CR-74 (1.5 %), *S. griseus* ♯1 (rarely) and *S. griseus* MC-33 (rarely), binucleate spores were observed.

3) Trinucleate spores were rarely observed only in *S. olivaceus* NRRL-B-1125.

4) Ring nucleus was observed in *S. olivaceus* GSM^{r}_{5000}

5) It was observed that there were so many none-nucleate spores in *S. olivaceus* CR-74.

The writers wish to express their sincere thanks to Prof. A. Yuasa of the University of Tokyo for his kind advice and suggestion. Thanks are also due to Mr. M. Furukawa for his assistance.

References

1) Kinoshita, S., and Itagaki, S., Bot. Mag. Tokyo 71: 335 (1958). 2) Breed, R. S., Murray, E. G. D., and Smith, M. S., Bergey's 'Manual of Determinative Bacteriology' (1957). 3) Klieneberger-Nobel, E., J. Gen. Microbiol. 1: 22 (1947).

摘　　要

第 1 報にひきつづき，B 群に属する 13 株および C 群に属する 18 株の観察をおこない，その結果を報告した。

1. B 群について。

(1) 7 株はほとんど完全に単核である。

(2) 4 株，すなわち，*S. lavendulae* ATCC-8664 (17 %), *S. erythrochromogenes* W-115C (8%), *S. pheochromogenus* W-41 (3 %) および *S. sinnamonenisis* (稀) において 2 核を認めた。

(3) *S. lavendulae* ATCC-8664 において，まれに 3 核を認めた。

(4) *S. roseochromogenus* において，明瞭な核を観察することができなかつた。

2. C 群について。

(1) 12 株はほとんど完全に単核である。

(2) 6 株，すなわち，*S. olivaceus* NRRL-B-1125 (12 %), *S. microflavus* 13-A (8 %), *S. sahachiroi* (5 %), *S. olivaceus* CR-74 (1.5 %), *S. griseus* ♯1 (稀) および，*S. griseus* MC-33 (稀) において 2 核を認めた。

(3) *S. olivaceus* NRRL-B-1125 においてのみ 3 核を認めた。

(4) *S. olivaceus* GSM^{r}_{5000} において，環状様核を観察した。

(5) *S. olivaceus* CR-74 において，多数の無核胞子が認められた。

Biochemical and Genetical Investigations of Flower Color in Swiss Giant Pansy, *Viola* × *Wittrokiana* Gams.*

II. Chromatographic Studies on Anthocyanin Components**

by Toru ENDO***

遠藤　徹***：パンジーの花色の遺伝生化学的研究　II. クロマトグラフ法によるアントチアニン成分の研究

Received October 29, 1958

In a preceding paper of this series[1], it was shown that five anthocyanin derivatives belonging to cyanidin and delphinidin groups are present in flowers of Swiss Giant pansy. In our approach to the genetic background of flower color variations, it is of particular importance to determine the type of aglycone and also of glycoside as regards individual anthocyanin components appearing in flowers, since several important biochemical steps, such as glycosidation, hydroxylation and methylation of anthocyanidin molecule, are known to be controlled by genes.

According to the methods established by Bate-Smith[2),3),4)], Hayashi[5~8)] and his coworkers, the present author has carried out the detailed examination of anthocyanin components which were separated paper-chromatographically from the flowers of several pansy varieties.

Materials and Methods

Materials. Among five cyanic varieties of Swiss Giant pansy, deep red (Alpenglow) and deep purple (Berna) varieties were mainly used as materials. The flowers of these varieties comprise a good source of anthocyanins, and also contain most of the pigment constituents found in other varieties, as reported previously.

Preparation of anthocyanins. For the preparation of anthocyanin mixtures available for the chromatographic analysis, ca. 2 g. of fresh petals were immersed in 1 % cold methanolic hydrochloric acid for 2 hours and filtered. The filtrate was concentrated *in vacuo* up to a syrup at 30°. Besides, sugar-free and organic acid-free samples were prepared as follows: ca. 300 g. of fresh petals were macerated in a blendor with 0.5 % aqueous hydrochloric acid and filtered. Pigments were precipitated from the

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* This is the correct specific name which was proposed in 1925 by H. Gams as a collective designation of cultivated pansies. The name, *Viola tricolor* L. used in my previous paper, should be replaced by this name.

** Contribution No. 261 from the National Institute of Genetics.

*** National Institute of Genetics, Misima, Sizuoka-ken, Japan. 国立遺伝学研究所。

filtrate as lead salts, and recovered as chloride by dissolution in dry 7 % methanolic hydrochloric acid, and precipitated as dark red matter by the addition of anhydrous ether.

Paper chromatography. One-way paper chromatography was applied for the separation of individual pigments as well as sugar and organic acid components. The solvents employed are as follows:

Designation	*Composition*	*Ratio (v/v)*	*Applied for*
BuOH-HCl-1	*n*-butanol/conc. hydrochloric acid/water	5:1:2	anthocyanin
BuOH-HCl-2	*n*-butanol/conc. hydrochloric acid/water	5:1:4	anthocyanin
HFo-HCl	80 % formic acid/conc. hydrochloric acid/water	5:1:4	anthocyanidin
BuOH-Prd	*n*-butanol/pyridine/water	6:3:1	sugar
BuOH-AmPh	*n*-butanol saturated with 0.5 N *sec.* ammonium phosphate	—	organic acid

Spraying reagents. Sugar spots on the chromatogram were detected by spraying with 1 % ethanolic solution of aniline hydrochloride[9] followed by heating for 5 min. at about 110°. Spots of organic acids were developed by spraying with a mixture of equal volume of 1 % ethanolic solution of methyl red and citrate-phosphate buffer solution of pH 7.0.

Isolation of anthocyanins. Since the column chromatographic separation of anthocyanin components has not been accomplished in satisfactory manner[10], the isolation was made by means of mass paper-chromatography throughout, and almost pure samples could be obtained as follows: 1 % methanolic hydrochloric acid solution of anthocyanins was streaked on every ten sheets of Tôyô No. 50 filter paper (40×40 cm.) and irrigated with the solvent, BuOH-HCl-1 or BuOH-HCl-2. The anthocyanin bands obtained were separated by cutting and eluted with 5 % acetic acid in methanol. After concentration of the combined eluates *in vacuo*, the resultant syrup was used for several tests.

Saponification. Acylated anthocyanins are characterized in general by giving the spots of high Rf-values accompanied by conspicuous tailing. In view of these behaviors, the flower materials examined seemed to contain at least two acylated anthocyanins, so that it was necessary to be studied further by means of saponification test.

For this purpose, each of the anthocyanins separated as above was treated for 2 hours with 10 % methanolic potassium hydroxide in an atmosphere of hydrogen. The solution was acidified with anhydrous 7 % methanolic hydrochloric acid, and the saponified anthocyanin was precipitated together with a large amount of potassium chloride by the addition of dry ether. From the precipitates, the anthocyanin was extracted thoroughly with methanol and chromatographed.

For the test of organic acid residues, a remaining portion of each anthocyanin fraction was treated with 10 % aqueous potassium hydroxide in an atmosphere of

hydrogen for 2 hours. After acidification with aqueous hydrochloric acid, the solution was repeatedly extracted with ether. The combined ethereal solution was evaporated to dryness, and the residues were applied for chromatographic test.

Hydrolysis. The anthocyanin was boiled in 10 % hydrochloric acid for 10 min., and the resultant aglycone was extracted with *iso*-amyl alcohol and chromatographed. The aqueous layer, if necessary, was passed through a resin column (1.5×10 cm.) filled with Amberlite IRC-120 to remove traces of anthocyanins. Colorless aqueous layer was repeatedly passed through Amberlite IRA-410 resin column, whereby hydrochloric acid was removed almost completely. Deionized solution from the column was concentrated *in vacuo* at ca. 40° and applied to chromatography of sugars.

For the determination of the position of sugar attachment in anthocyanins, partial hydrolysis was carried out as follows: both isolated and authentic anthocyanins were heated in parallel with 12 % ethanolic hydrochloric acid at 70° on a water bath. During hydrolysis, every small portion was pipetted out at regular intervals, and spotted side by side on a filter paper and chromatographed.

Results

Determination of aglycones. From the present paper-chromatographic study, it was found that at least six kinds of anthocyanins are present in the flowers of pansy varieties. These anthocyanins are designated tentatively as aC_1, aD_2, C_3, D_4, C_5 and D_6, of which the last one was newly found. Among them, aD_2 and C_3 predominate in bluish and reddish varieties in general. Corresponding aglycones are carefully compared with each other on the chromatogram (Table 2). The results indicate that aC_1, C_3 and C_5 belong to cyanidin-, and aD_2, D_4 and D_6 to delphinidin-group. Therefore, these anthocyanins were co-chromatographed further with authentic cyanidin- and delphinidin-derivatives, respectively. The results are summarized in Tables 3 and 4, from which it may be concluded that C_3 is identical with keracyanin (antirrhinin), whereas the others agree with none of the known anthocyanins.

Table 1. Rf values of pansy anthocyanins isolated and mixed. (Temp. 25°)

Sample	Solvent	aC_1	aD_2	C_3	D_4	C_5	D_6
Isolated	BuOH HCl-1	0.84	0.78	0.60	0.40	0.42	0.14
Mixed	BuOH-HCl-1	0.84	0.72	0.59	0.43	0.31	0.18
Mixed	BuOH HCl-2	0.55	0.39	0.28	0.17	0.12	0.07
Isolated	HFo-HCl	0.79	0.74	0.67	0.58	0.80	0.60
Mixed	HFo-HCl	about 0.80 (not separable)					

Table 2. Rf values of authentic anthocyanidins and of the pigments from pansy.

Anthocyanidin	HFo-HCl	Sample from	HFo-HCl
Pelargonidin	0.36	aC_1	0.22
Peonidin	0.30	C_3	0.22
Malvidin	0.27	C_5	0.22
Cyanidin	0.22	aD_2	0.12
Petunidin	0.18	D_4	0.12
Delphinidin	0.12	D_6	0.12

Table 3. Rf values of cyanidin-glycosides. (Temp. 25°)

Anthocyanin	Glycoside type	BuOH-HCl 1	HFo HCl
Chrysanthemin	3-glucoside	0.50	0.53
Idaein	3 galactoside	0.48	0.53
Keracyanin	3-glucorhamnoside	0.62	0.67
Ilicicyanin	3-glucoxyloside	0.62	0.74
Cyanin	3:5-diglucoside	0.32	0.68

Table 4. Rf values of delphinidin-glycosides. (Temp. 25°)

Anthocyanin	Glycoside type	BuOH HCl 1	HFo HCl
Unnamed	3-glucoside	0.29	0.40
Empetrin	3-galactoside	0.27	0.42
Hyacin	3 glucoglucoside	0,19	0.57
Delphin	3:5-diglucoside	0.18	0.57

Determination of sugar and organic acid components. So far as is known, organic acids attached to the anthocyanin molecule in general are *p*-coumaric (*p*-hydroxycinnamic), malonic and *p*-hydroxybenzoic acid, and sugar components are known to be rhamnose, xylose, arabinose, glucose and galactose.

Therefore, the authentic specimens of these compounds were used as standard in chromatographic evaluation of the components of anthocyanins in question. Followingly, it becomes evident that both aC_1 and aD_2 are associated with *p*-coumaric acid (Tables 5 and 6), and that the sugar components are glucose and rhamnose in all six anthocyanins examined (Table 7).

Table. 5. Rf values of pansy anthocyanins after saponification.

Sample	BuOH-HCl-1	HFo-HCl
Saponified aC_1	0.26	——
Saponified aD_2	0.14	0.61

Table 6. Rf. values of organic acids.

Organic acid	BuOH-AmPh
p-Coumaric acid	0.49
p-Hydroxybenzoic acid	0.61
Malonic acid	0.02
Sample from aD_2	0.49
Sample from a mixture of aC_1 and aD_2	0.50

Table 7. Rf values of sugars.

Sugar	BuOH-Prd	Sample from	BuOH-Prd	
Rhamnose	0.61	aD_2	0.60	0.26
Xylose	0.44	C_3	0.61	0.26
Arabinose	0.37	D_4	0.61	0.25
Glucose	0.27	C_5	0.61	0.26
Galactose	0.22	D_6	0.59	0.27

Determination of sugar residues. Prior to detailed examination of unknown anthocyanins from pansy, partial hydrolysis of the authentic cyanin chloride was carried out under almost the same experimental conditions as described by Hayashi *et al.*[7].

In this experiment, three kinds of hydrolytic product, namely chrysanthemin, cyanenin and cyanidin, were detected on a chromatogram, as shown in Fig. 1(a). The Rf value of cyanenin was found to be higher than that of chrysanthemin on irrigation with the solvent, BuOH-HCl-1, whereas two cyanidin-monoglucosides, chrysanthemin and cyanenin, were not separable with each other after irrigation with the solvent, HFo-HCl, as shown in Fig. 1 (b).

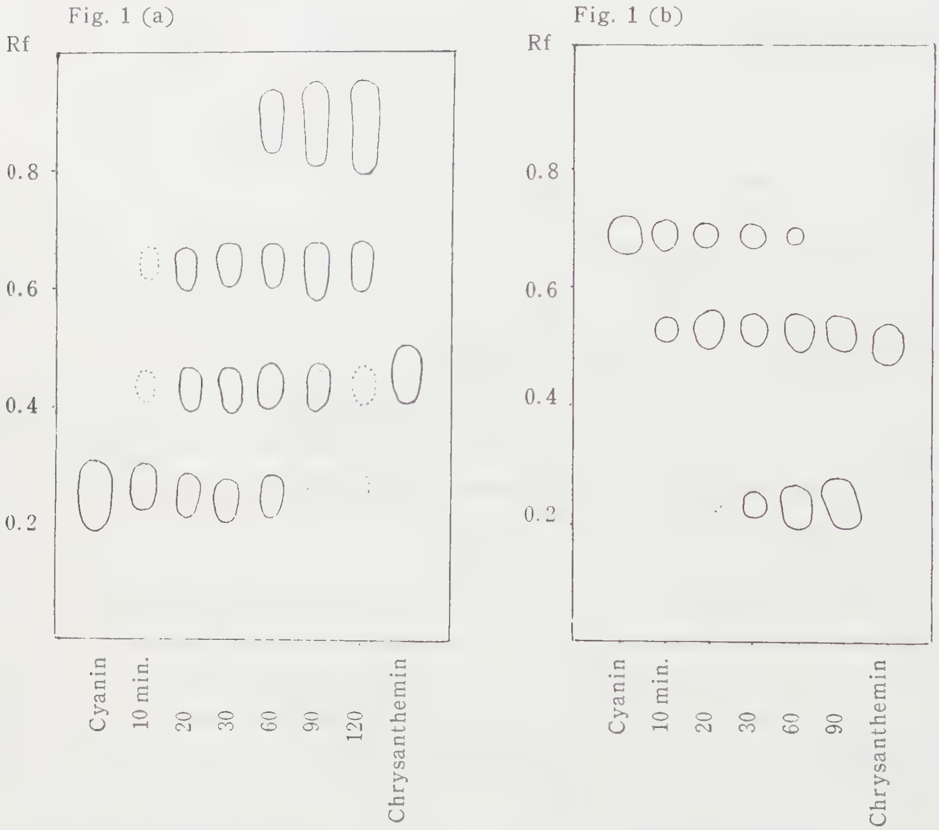

Fig. 1. Paper chromatogroms of the products of partial hydorlysis of cyanin. Developed with (a) BuOH-HCl-1; (b) HFo-HCl.

As regards the anthocyanins from pansy, aD_2, C_3, D_4 and C_5, were examined in similar manner using delphin, hyacin, delphinidin-3-glucoside, cyanin and chrysanthemin as standards.

From the examination of organic acid as well as sugar components, it is suggested that aD_2 is *p*-coumarylglucorhamnosyl-delphinidin. On partial hydrolysis of the anthocyanin, three normal spots, Rf 0.12, 0.25 and 0.36, together with one tailing spot, Rf ca. 0.66, appeared on the chromatogram, as shown in Fig. 2 (a). The tailing spot is obviously delphinidin, which remains until the latest stage of treatment. The spot, Rf 0.12, which appears at an early stage of treatment, is certainly responsible for glucorhamnosyl-delphinidin which is derived from the original compound after libe-

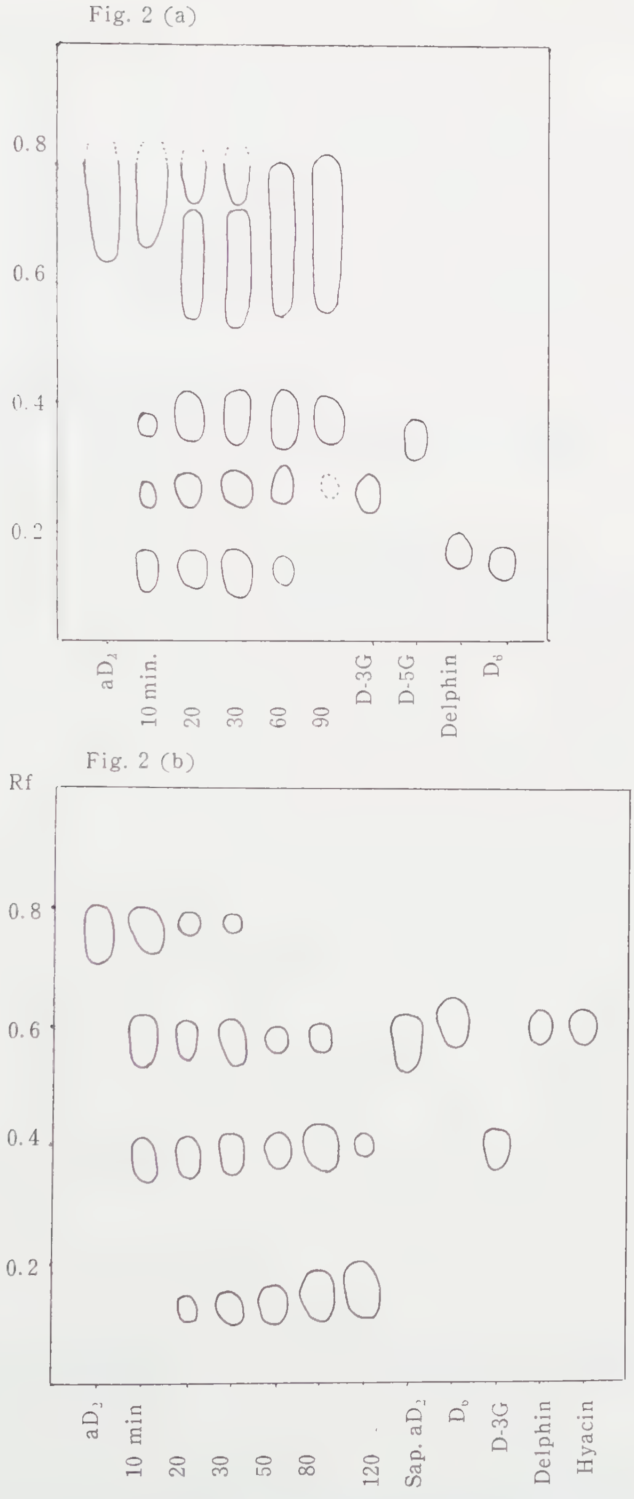

Fig. 2. Paper chromatograms of partial hydrolysis of aD_2. Developed with (a) BuOH-HCl-1; (b) HFo-HCl. D-3G=delphinidin-3-glucoside Sap. aD_2=saponified: product of aD_2.

ration of *p*-coumaric acid. The other two, Rf 0.25 and 0.36, correspond to delphinidin-3-glucoside and -5-glucoside, respectively, which are obtainable also by partial hydrolysis of delphin chloride. Although another possibility that one of them is delphinidin-rhamnoside is not excluded, this could not be ascertained owing to the lack of a specimen available for comparison. In consequence, it is suggested that aD_2 is not violanin[11] (delphinidin-3-*p*-coumarylglucorhamnoside), which has been commonly isolated from bluish purple flowers of cultivated pansies, but one of the following four anthocyanins: delphinidin-3-*p*-coumarylglucoside: 5-rhamnoside, -3-glucoside: 5-*p*-coumaryl-rhamnoside, -3-*p*-coumarylrhamnoside: 5-glucoside and -3-rhamnoside: 5--*p*-coumarylglucoside.

Figs. 3 (a) and 3 (b) represent the chromatograms showing two kinds of hydrolytic product formed on partial hydrolysis of C_3. One of them corresponds to chrysanthemin and the other to cyanidin. Accordingly, it is concluded that C_3 is cyanidin-3-glucorhamnoside.

The result on D_4 is very similar to that on C_3. The two products obtained by partial hydrolysis of D_4 correspond to delphinidin and its 3-glucoside, respectively, in chromatographic behavior, as shown in Fig. 4. The sugar components of D_4 were

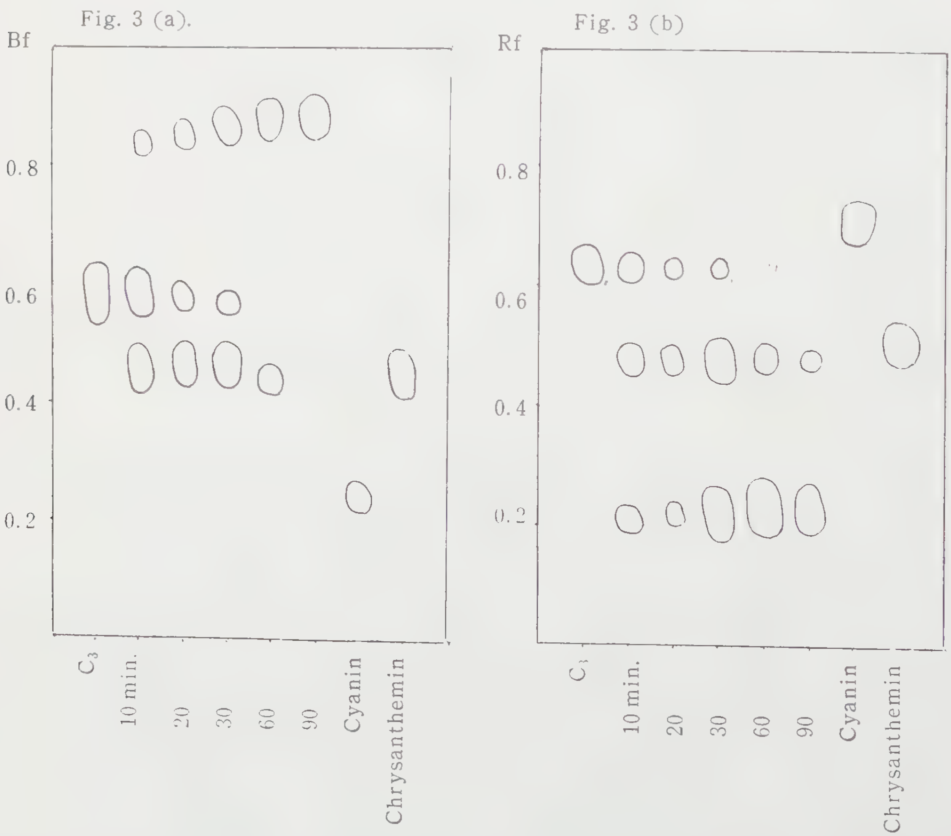

Fig. 3. Paper chromatograms of the products of partial hydrolysis of C_3. Developed with (a) BuOH-HCl-1; (b) HFo-HCl.

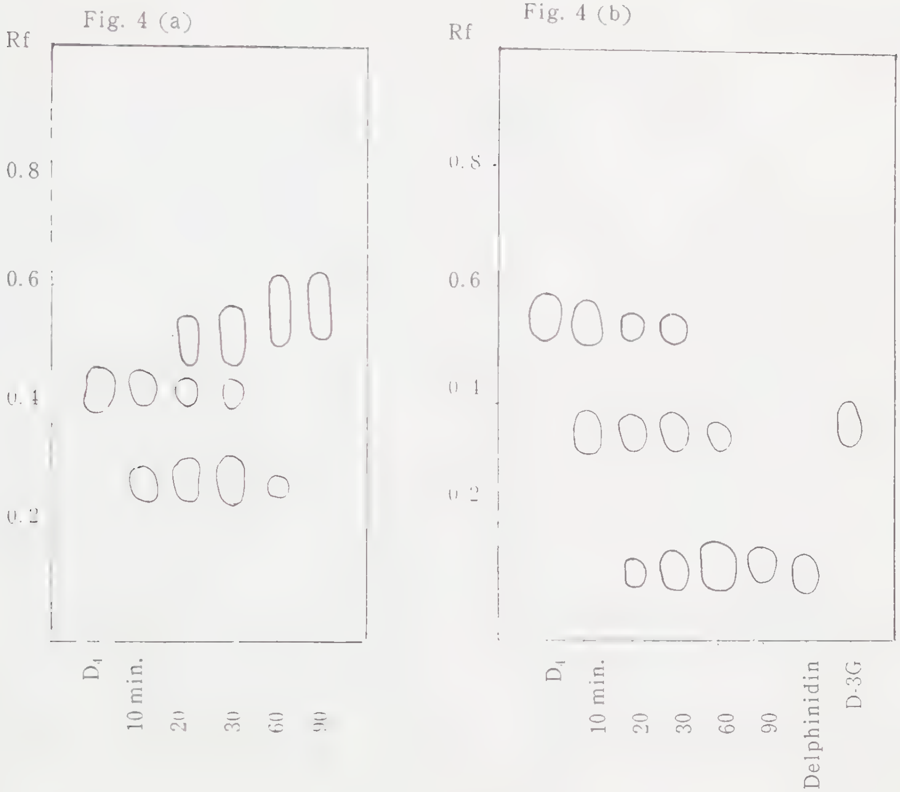

Fig. 4. Paper chromatograms of the products of partial hydrolysis of D_4. Developed with (a) BuOH-HCl-1: (b) HFo-HCl.

identified as glucose and rhamnose. Therefore, D_4 must be delphinidin-3-glucorhamnoside (tulipanin)[12], which was newly isolated from dark purple flowers of *Tulipa gesneriana*.

On partial hydrolysis of C_5, four kinds of hydrolytic product appeared on the chromatogram, as shown in Fig. 5 (a). Their Rf values are 0.25, 0.42, 0.64 and 0.88, respectively. The last one corresponds to cyanidin. The spot, Rf 0.25, which appeared at an early stage of hydrolysis, is proved to be cyanin by co-chromatography. The remaining two spots, Rf 0.42 and 0.64, correspond to chrysanthemin and cyanenin, respectively. Thus, C_5 is cyanidin-triglycoside, which is probably cyanidin-3-glucorhamnoside : 5-glucoside or -3-glucoside : 5-glucorhamnoside.

Determination of sugar residues of aC_1 and D_6 is still in progress. At present, it is not probable that aC_1 is an acylated type of C_3 or C_5, and D_6 may be non-acylated type of aD_2, because both anthocyanins have identical sugar components and very similar Rf values, as shown in Fig. 2.

Preliminary identification of anthocyanins. For the identification of pansy anthocyanins, it is necessary to assume the generality of the following facts :

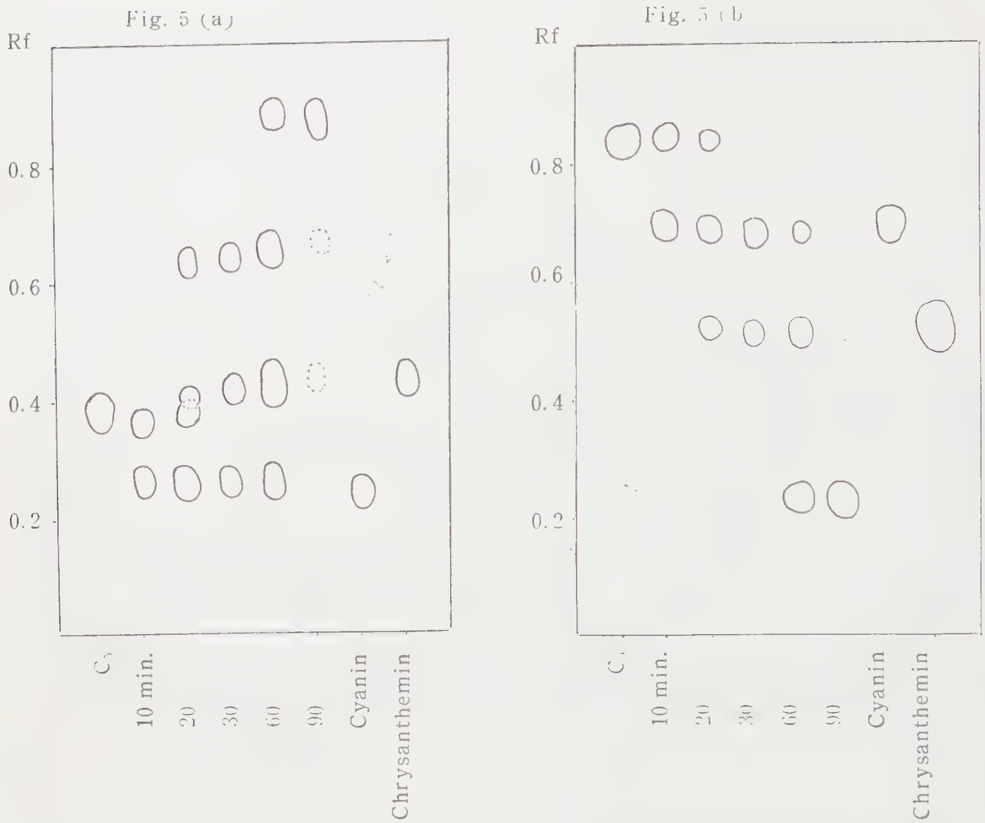

Fig. 5. Paper chromatograms of partial hydrolysis of C_5. Developed with (a) BuOH-HCl-1; (b) HFo-HCl.

(1) Attachment of sugar residues is restricted to 3- and 5-positions in natural anthocyanins.

(2) During the course of partial hydrolysis of a given anthocyanin, the sugar and organic acid components are liberated, one by one, from the original anthocyanin.

(3) Organic acids which combine with sugar hydroxyls of anthocyanin are liberated by acid hydrolysis.

(4) Rf values are apt to change within the range of ca. ±0.02 in general.

In relation to the subject (3), it is noteworthy that Karrer *et al.* have made an interesting observation, that in salvianin [3-di(methylmalonyl): 7-*p*-coumaryl-pelargonin][13),14)], coumaric acid residue is liberated not by acid hydrolysis, but only by treatment with alkali[13)]. From this fact, they assumed that organic acids which can be easily liberated by acid treatment combine with sugar hydroxyls of anthocyanins. If this is true, it may be that (3) provides an effective clue for the discrimination of the position of organic acid attachment in the anthocyanins examined here.

Under these considerations, following formulations may tentatively be assigned to the six anthocyanins found in flowers of Swiss Giant Pansy:

aC_1: cyanidin-?-*p*-coumarylglycoside,
aD_2: delphinidin-3:5-*p*-coumarylglucorhamnoside,
C_3: cyanidin-3-glucorhamnoside (keracyanin),
D_4: delphinidin-3-glucorhamnoside (tulipanin),
C_5: cyanidin-3:5-glucoglucorhamnoside,
D_6: delphinidin-?-glucorhamnoside.

Summary

In the common garden varieties of Swiss Giant pancy, *Viola Wittrockiana* Gams, the flower colors ranging from red to blue are caused by at least six anthocyanins. They were separated from each other by mass paper chromatography in comparatively pure state. Their component sugar, organic acid, etc. were studied chromatographically after complete as well as partial hydrolysis, and also after saponification with alkali. The results show that the major pigment is keracyanin in reddisch varieties, whereas the pigment in bluish varieties is not violanin (delphinidin-3-*p*-coumarylglucorhamnoside) but a new anthocyanin, probably having a constitution of delphinidin-3: 5-*p*-coumarylglucorhamnoside. Among four kinds of minor constituent, two are shown to be tulipanin and cyanidin-3:5-glucoglucorhamnoside, and the remaining two are derivatives of cyanidin and delphinidin.

Acknowledgment

The author wishes to express his sincere thanks to Professor K. Hayashi, of Tokyo University of Education, for his kind guidance and advice and for the supply of all authentic anthocyanins and organic acids which made this investigation possible.

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摘　　要

栽培型パンジーの一系統スイスジャイアントの花色変異には，6 種類のアントチアニンが関係している。これをペーパークロマトグラフィーで分離して，比較的純粋なアントチアニンを得た。これらについて，それぞれ鹸化，完全および部分的加水分解を行い，クロマトグラム上で規準標本と比較して同定を試みた。その結果，赤色花の主要色素は keracyanin であり，青色花のそれは delphinidin-3:5-*p*-coumarylglucorhamnoside であつて，violanin (delphinidin-3-*p*-coumarylglucorhamnoside) ではないようである。比較的少量含まれている色素の一つは tulipanin で，他は cyanidin-3: 5-glucoglucorhamnoside と推定された。他の 2 つは cyanidin および delphinidin の誘導体である。

雑　録

植物学研究連絡委員会報告

委員長　松浦　一　　幹　事　田中信徳

委　員　芦田譲治，芳賀　忞，原　寛
服部静夫，神谷宣郎，木村有香
松浦　一，三輪知雄，門司正三
小倉　謙，大政正隆，小野記彦
島村　環，下斗米直昌，白石代吉
田杉平司，田中信徳，和田文吾

植物学研究連絡委員会が昭和 32～33 年に行なった主な事柄は次の通りである。

1. 自然史科学研究センターの設立がＪＳＣ総会で議決された（4月）。
2. 第4部に海洋学研連ができ，植物部門から新崎盛敏氏が委員となった（5月）。
3. 第三次南極観測隊の生物班員に福島博氏（横浜市立大）を推薦した（6月）。
4. ダーウィン進化論100年記念事業として，11月1, 2, の両日ＪＳＣ講堂でシンポシウムと講演会を行なった。

　11月1日（土）午前シンポシウム
　　座　長　岡田　要，長谷部言人
　　分類学　前川文夫，高島春雄
　　古生物学・人類学　高井冬二，鈴木尚
　11月1日（土）午後講演会
　　ダーウィン進化論100年記念について　篠遠喜人
　　古生物と進化　早坂一郎
　　個体発生と系統発生　丘英通
　　生態学と進化　吉井義次
　　遺伝進化学　森脇大五郎
　11月2日（日）午前シンポシウム
　　座　長　丘英通，木原　均
　　発生学　山田常雄，石田寿老
　　栽培飼養のもとにおける進化　山田行雄，根井正利
　11月2日（日）午後シンポシウム
　　座　長　横山忠雄，森脇大五郎，宮地伝三郎，須田昭義
　　生態学　沼田真，川村智治郎
　　遺伝進化学　倉林正尚，木村資生
　　進化の問題点　永野為武，木村陽二郎，徳田御稔

5. Jap. Journ. Botany の編集委員と幹事を次の諸氏に委嘱し，Vol. 17, No. 1 を編集した（10月）。

　編集委員　原　寛，服部静夫，木村有香
　　　　　　松浦　一，前川文夫，門司正三
　　　　　　小倉　謙，大槻虎男，田中信徳
　幹　　事　原　寛，田中信徳

6. 1959年8月19～29日，モントリオールで開かれる IX International Botanical Congress に派遣する代表の候補者を各委員からの投票および直接委員長に連絡のあった22名（氏名略）について，関係資料を参考として審議し，Ⓐ国費によるもの：①服部静夫，②神谷宣郎，③大槻虎男，④三木茂，⑤田宮博，⑥宝月欣二，⑦前川文夫，Ⓑ私費によるもの：①和田文吾，②神保忠男，③服部新佐，④山田幸男（敬称略，数字は順位）の11名をそれぞれＪＳＣに推薦することを決定した。

　また Nomenclature 会議への代表候補者として①北村四郎，②原寛の2名をＪＳＣに推薦することを決定した。

　なお，会議の規模が大きい点，5年目に開かれる点，などを考慮し，できるだけ多数の代表を派遣できるよう，委員長が尽力することになった。

本　会　記　事

下郡山正巳氏が外遊されましたので，編集委員を林孝三氏にお願いいたしました。

関　東　支　部

○11 月例会（11 月 22 日（土），於東大・理・植）

伊藤太郎：アカパンカビの子実体形成，特に性ホルモン様物質の作用について。浜谷稔夫：ジンチョウゲ科の解剖と系統

○12 月例会（12 月 20 日（土），於東大・理・植）

湯浅明：アメリカみやげ—コウボキン細胞学。

中　部　支　部

○第 53 回例会（10 月 4 日（土），於名大・理・生）

須賀瑛文：愛知県の車軸藻科の生態について

岡崎令治：生細胞内ＤＮＡ合成の機作

外国論文抄錄の投稿について

1)　原稿は和文で，論文 1 篇につき 400 字づめ原稿用紙 2 枚以内とします。原著者名，題名，雑誌名，巻数，頁数および年次を明記すること

2)　抄録の採否は編集委員会が決定します。

3)　採用抄録 1 篇につき 300 円の稿料を出します。

原稿は日本植物学会編集幹事あてお送りください。

例：根端におけるプロトクロロフィル

Hejnowicz, Z., Protochlorophyll in root tips. physiol. plantarum, **11**: 878-888(1958).

Errata

Vol. 71, No. 844, p. 319-325.

誤	正
2×10^{-3}M	5×10^{-4}M
5×10^{-3}M	2×10^{-4}M
2×10^{-4}M	5×10^{-5}M
5×10^{-4}M	2×10^{-5}M
5×10^{-5}M	2×10^{-6}M

Vol. 71, Nos. 845 846.

p. 393

第 4 表　No. 8 の 3 列目の＋をとり 4 列目に＋を入れる

p. 397

第 7 表　第 1 列　上から 3 つ目の ECS を ECX に

第 2 列　上から 7 つ目の E を EX になおす。

p. 398

7 行目　Tubercle　のあとに bacillary を入れる。

評　議　員　選　挙

会則第 11 条および付則第 3 第 2 条により評議員の改選を行ないます。

〔会則抄〕

第 11 条　評議員は評議員会を構成する。評議員会は会長の諮問の範囲で本会の要務を審議し，また総会への提案を作る。

付則第 3　第 2 条　評議員は各支部別に支部会員によって選出される。その定員は各支部最低 2 名とし，会員数が 100 名を越える支部では 50 名までごとに 1 名を増す。評議員は任期を 3 期以上重任することはできない。なお会長および幹事長は評議員を兼任することができない

付則第 3　第 2 条によって下記の方々は現在 2 期つづけて評議員になっておられ，この度は評議員には選出されません。

北海道支部（定員 2 名）	山田幸男		
東北支部（定員 1 名）	木村有香		
関東支部（定員 8 名）	前川文夫	三輪知雄	亘理俊次
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近畿支部（定員 4 名）	芦田譲治	今村駿一郎	新家浪雄
中国四国支部（定員 3 名）	猪野俊平	下斗米直昌	堀川芳雄
九州支部（定員 2 名）	小島均		

先日お手許にお送りいたしました会長選挙用紙に御記入のうえ，**2 月末日**までに　東京都本郷局区内東京大学理学部植物学教室　日本植物学会選挙係まで，もれなくお送りください。

投稿の注意

1) 投稿は会員にかぎります。ただし，共同研究者はかならずしも会員にかぎりません。

2) 原稿は他に印刷されていない内容のものにかぎります。(文部省科学研究費による研究業績は，その旨明記してください。)

3) 原稿は簡潔に書き，原則として，すりあがり 8 ページ以内とします (すりあがり 1 ページは，和文では 400 字づめ用紙に 5 枚，欧文では一行に 60 字，27 行うったタイプライト紙で 2 枚ていどです)。なおとくに編集委員会で必要とみとめた場合は，制限ページ数をこえた分 1 ページにつき，1200 円の著者負担でのせることがあります。

4) 原稿の体裁：

a) 欧文 (英・独・仏・ラテン) 原稿は，かならず片面に一行あきに黒リボンをもちい，タイプライト印書してください。用紙は薄紙はさけて標準型 (約 30×21 cm. または A 4 版) をもちい，上下左右を 3—4 cm. あけてうち，コピーではなく正本をお送りください。

そのさい，原稿は，原則としてあらかじめそれぞれの語を常用している外国人にみせ，少くとも文法，文体上のあやまりのないことをたしかめてください。編集委員が必要とみとめ，投稿者の同意を得た場合は，編集委員会から適当な外国人に語学上の修正をもとめることがあります。この場合，外人への謝礼の実費を申しうけます。なお，和文摘要 (400 字づめの原稿用紙 2 枚以内) をつけ，和文題名を明記してください。

b) 和文原稿は，400 字づめ原稿用紙に横がきし，現代かなづかいをもちい，できるだけ当用漢字にし，欧語音訳には片かなを用いてください。学術用語は，文部省編，学術用語植物学編 (1958 年 4 月発行) を参考にしてください。なお，すりあがりが半ページ以内の欧文摘要をつけること。

c) 表題と著者勤務先は和欧両文でかいてください。

d) 数字はすべてアラビア数字をもちい，数量の単位はメートル法によること。欧文学名の下には——をつける。

e) 図表について：できるだけ小さくし，本文とは別の用紙にかくこと。図の説明も図とはべつにかき，本文中にいれる位置を明示し，そこに説明をはっておくこと。図はすりあがりでページ幅より小さい場合には横に少なくとも 4 cm. 以上あけて印刷します。図や文字の大きさ・太さは希望するすりあがりに対し，1.5～2 倍ぐらいにかくのが適当です。写真の図中にいれる文字は，印刷した活字をはりこむか，黒インクでていねいにかいてください (タイプライトしたものは不可)。図表の裏には著者名・表題・図表の番号をかいておくこと。

f) 文献引用形式：

引用文献はおわりに一括し，引用順に番号をつけてならべます。本文中では引用箇所に引用番号を 1)，2)，のように右にかきこんでください。文献引用の形式は著者名，雑誌名 (書名) 巻数，ページ数，年号の順に記し，雑誌巻数には～～の下線をつける。

(例) Ryan, F. J., and Beadle, G. W., Amer. J. Bot. 30: 784 (1943)

g) 活字指定は編集幹事がしますから，著者がとくに希望する箇所があれば，黒鉛筆で指定しておいてください。

5) 著者校正は初校だけにかぎります。返送のさいは，2 日以内に原稿とともに書留速達で送ってください。なお，原稿は著者校正でその内容に補正を加える必要のないほど，投稿のさいにじゅうぶん検討してください。もし，やむを得ず校正でその内容に重大な変更を加えるときは，受けつけの日づけを延期されることになります。

6) 速報は緊急発表の必要なものにかぎり，すりあがりで 1 ページ以内のものとし，1500 円をそえて投稿のこと。

7) 原稿の採否，修正，登載の順序はすべて編集委員会の決定によります。校正および文体の統一は原則として編集幹事に一任してください。

8) 別刷りは 50 部進呈し，それ以上は著者の実費負担ですから，必要の総部数を原稿に明記してください。

9) 上記の注意にしたがわないでかかれた原稿は返却されることがあります。また原稿の体裁を学会が修正することがありますが，その場合に必要な費用は著者に実費負担していただきます。

10) 原稿は下記あて，かならず書留便で送ってください。

東京都本郷局区内
東京大学理学部植物学教室内
日本植物学会編集幹事

11) 原稿は図表とともに雑誌発行ののちにお返しします。

日本植物学会会則

（昭和 31 年 7 月 14 日改正）

第 1 条　本会は日本植物学会という。

第 2 条　本会は植物学の進歩と普及をはかり，あわせて会員たがいのしたしみを増すことを目的とする。

第 3 条　本会は前条の目的を達するために「植物学雑誌」そのほかの出版物の刊行，大会・講演会・講習会などの開催，そのほか必要と思われる事業を行なう。

第 4 条　本会の会員は次の 5 種とする：

　通常会員・終身会員・特別会員・外国通信会員・名誉会員

第 5 条　通常会員とは所定の会費を納めるものをいい，終身会員とは所定の終身会費を納めたものをいう。

第 6 条　特別会員とは引続き本会の会員であって本会に対していちじるしい功労のあった者，外国通信会員とは本会に関係の深い外国人，また名誉会員とは植物学に対して功労のいちじるしい者で，いずれも評議員会で協議し会長が総会で推薦し承認された者をいう。ただしやむを得ない場合は，あとで総会の承認を求めることがある。これらの会員は会費を要しない。

第 7 条　本会には地方支部を置き，会員はいずれかの地方支部に属するものとする。地方支部についての規定は別に設ける。

第 8 条　本会には次の役員を置く：

　会長：1 名　幹事長：1 名　幹事：若干名

　評議員：若干名　編集委員：若干名

第 9 条　役員は会員の中から選出し，任期は 2 カ年とする。ただし重任することができる。選出についての規定は別に設ける。

第 10 条　会長は会務の全体をすべる。幹事長は会長を助けて会務を処理する。幹事は庶務・会計・編集・図書管理など日常の会務を行なう。

第 11 条　評議員は評議員会を構成する。評議員会は会長の諮問の範囲で本会の要務を審議し，また総会への提案をつくる。

第 12 条　編集委員は編集委員会を構成する。幹事長はその長となる。編集委員会は「植物学雑誌」の編集に関しての一切の責任を負う。

第 13 条　本会の会計年度は 1 月に始まり 12 月に終る。

第 14 条　本会は原則として毎年 1 回総会を開き，会務を協議し議決する。なお会長が必要と認めた場合には臨時総会を開くことができる。

第 15 条　本会は総会の時大会を開き研究発表などを行なう。大会には大会会長そのほか若干名

（裏面へつづく）

……………………きりとり線……………………

入会申込書

氏名 ふりがな	男　女 明治 大正　年　月　日生 昭和	この紙を切りとって所要の事がらを記入または○でかこみ会費をそえて学会あてにお送り下さい。どなたでも入会できます。
勤務先 （所在地）		
住所		

通常 終身　会員に	昭和…………年　　から	……………………支部へ	雑誌の送り先を指定して下さい。希望する方へ○印を

入会の申込，会費（年 900円）の払込，そのほか会へのご連絡のあて先は：

東京都文京区東京大学理学部植物学教室内　日本植物学会です。

それから会費の払込は振替貯金口座東京 11190 番を利用されるのがもっとも確実です。なお振替でお払込の場合は特に領収書をさし上げませんからあしからず。

の臨時の役員を置くことができる。大会会長は会長が推薦し，そのほかの役員は大会会長が依嘱する。

第 16 条　会員は会合に出席して講演をし議事に参加し，「植物学雑誌」に投稿し，また本会所有の図書を閲覧することができる。また毎号無料で「植物学雑誌」の配布を受ける。

第 17 条　会員が退会しようとするときは，そのことを本会に通知しなければならない。もし会費の滞納があるときはそのさい全額を納めなければならない。ただしすでに納めた会費は一切これを返さない。通常会員が会費を滞納したときはただちに前条の権利を停止し，1 ヵ年以上滞納したときは除名することがある。

第 18 条　本会の会則または付則を変更するには総会または臨時総会でこれを協議し，出席会員の 3 分の 2 以上の同意を得なければならない。

付則第 1　会　　費 （会則第 5 条関係）

第 1 条　通常会員の会費は年 900 円とし 300 円ずつ分納することも できる。終身会費は 15,000 円とする。

このほか国外在住会員に限り植物学雑誌の送料を負担する。

第 2 条　評議員編集委員以外の役員は在任中会費を要しない。

付則第 2　地方支部 （会則第 7 条関係）

第 1 条　地方支部は原則として 50 名以上の会員のある地方に置き，北海道・東北・関東・北陸・中部・近畿・中国四国・九州の八支部とする。

第 2 条　会員は居住地の支部に入るのが原則であるが，事情により他の支部に属することもできる。

第 3 条　支部には支部長を置く。支部長は支部を代表する。

第 4 条　そのほかの規定は各支部ごとに設ける

付則第 3　役員の選出方法 （会則第 9 条関係）

第 1 条　会長は全会員の投票で選出される。その際評議員会は若干名の候補者を推薦することができる。

第 2 条　評議員は各支部別に支部会員によって選出される。その定員は各支部最低 2 名とし，会員数が 100 名を越える支部では 50 名までごとに 1 名を増す。評議員は引続き 3 期選出されることはできない。なお会長および幹事長は評議員を兼任することができない。

第 3 条　幹事長・幹事・編集委員はいずれも会長が依嘱する。

……………………………………………… きりとり線 ………………………………………………

入会や転居などの場合には必ず本部のほかに支部へも連絡して下さい。支部のあて先は次のとおりです。なおどの支部へ入るかは大体地理的にきまるわけですが，ご都合でA支部よりもB支部の方が便利だという方はB支部に入られてもよいことになっています

北海道支部　札幌市北 8 条西 5 丁目　北海道大学理学部植物学教室
東 北 支 部　仙台市片平町　東北大学理学部生物学教室
関 東 支 部　東京都目黒区駒場町　東京大学教養学部生物学教室
北 陸 支 部　金沢市仙石町　金沢大学理学部植物学教室
中 部 支 部　名古屋市千種区不老町　名古屋大学理学部生物学教室
近 畿 支 部　京都市左京区北白川　京都大学理学部植物学教室
中国四国支部　広島市東千田町　広島大学理学部植物学教室
九 州 支 部　福岡市箱崎　九州大学理学部生物学教室

日本植物学会会員名簿

（支部別）（アイウエオ順）

（昭和 33 年 12 月 31 日現在）

名誉会員　○特別会員　外国通信会員　終身会員

1. 北海道支部

秋山茂雄	北大理植・同市北28条東3丁目
明峯俊夫	北大理植・札幌市南6条西9丁目 1018
朝比奈英三	札幌市北8条　北大低温科学研究所・札幌市南5条西 24 丁目
浅利政俊	北海道松前郡松前町立清部小学校・同町字清部 444
○伊藤誠哉	北大農植
伊藤太郎	帯広市外帯広畜産大生
稲垣貫一	旭川市北門町9丁目　北海道学芸大学旭川分校生物学教室
井上博	札幌市琴似町川添西　琴似小学校
井上藤二	札幌南高校・札幌市南 11 条西1丁目 14
石川鉱	北大理植・札幌市北 13 条東2丁目　銀玲荘内
岩塚佐吉	札幌市北 16 条西2丁目　藤女子短大
入倉幸雄	北海道農事試験場作物第四研究所・北海道千歳郡恵庭町字島松
宇佐美正一郎	北大理植・札幌市北 26 条東4丁目
梅沢京子	北海道雨竜郡深川町　深川東高校
大谷吉雄	北大理植・北海道札幌郡琴似町発寒 58
大野林三郎	北大理植
大淵亮二	函館市千代ケ袋町 68
大山正	函館市高岡町 9　市立弥生小学校
岡沢養三	北大理植・札幌市南 17 条西 12 丁目
岡本幹二	室蘭市小橋内町 1
笠巻明子	北大理植
川嶋昭二	札幌市北3条西5丁目　北海道・水産部・水産課　札幌市大通西 22 丁目
川代善一	北見市清見町　北見営林局計画課・同市同町
川端清策	岩見沢市　北海道学芸大岩見沢分校・空知郡三笠町幌内北区
木村卓郎	札幌市南 20 条西7丁目
北本毅	滝川市字東滝川　滝川東栄高等学校
倉林正尚	函館市八幡町　北海道学芸大学函館分校
桑山弥寿男	北大理植
河野昭一	北大農植・札幌市北7条西8丁目　昭井方
小松泰造	芦別市　芦別市立芦別中学校
*斎藤実	札幌市北海道教育委員会・学校教育課
酒井昭	札幌市北8条　北大低温科学研究所
阪井与志雄	北大理植・札幌市北4条西 22 丁目
佐々木茂	釧路市大川町 48　道立水試釧路支場
佐々木昭治	北大理植・札幌市南 26 条西8丁目
佐々木太一	北海道石狩国安足間　愛山中学
佐藤清左衛門	北海道夕張郡栗山町　王子製紙工業材木育種研究所
佐保貴	北大理植・札幌市南 18 条西9丁目　木村方
沢田義康	旭川市北海道学芸大旭川分校生物・同市春光町学芸大第三職員寮
菅原繁蔵	函館市千代ケ岱町 92-1
田川隆	北大農植・札幌市南 15 条西5丁目 1
田沢伸雄	北大理植・小樽市末広町 41
舘脇操	北大農植・札幌市北4条西 13 丁目
田中瑞穂	釧路市城山町　北海道学芸大釧路分校・同市鶴ケ岱 9
田辺至	北海道留萌市千鳥町　留萌高校
辻寧昭	北大理植・札幌市南 23 条西8丁目
辻井達一	北大農植・札幌市大通り西 27 丁目　小山田方
寺岡宏	北大理植
照本勲	札幌市北8条　北大低温科学研究所・同市北8条西 16 丁目
時田郇	函館市港町 253　北大水産・同市松陰町 10

土橋慶吉　江別市字西野幌 582　酪農学園短大図書館

豊国秀夫　北大理植・札幌市南2条西5丁目 31

仲尾滋子　北大理植・札幌市南7条西 17丁目

長尾正人　札幌市北 27 条東3丁目　北大官舎

中野　実　札幌市豊平5条 13 丁目　林試札幌支場豊平分場

中村義輝　室蘭市舟見町　北大理学部海藻研究所

西山保直　北大農植

長谷川由雄　北海道余市郡余市町　北海道区水産研究所・同町沢町 114

原　秀雄　北大農植物園・札幌市北2条西9丁目

樋浦　誠　北海道江別市西野幌

福田一郎　北大理植・札幌市北4条西 11丁目　東晃方

福原英司　北海道余市郡余市町　北海道区水産研究所

舟根　昇　札幌市琴似町　日本新薬札幌支店・同町 24 軒南

舟橋説往　北大理植・札幌市宮の森 544

堀　七郎　釧路市城山町　北海道学芸大釧路分校・同市城山町 139

前田喜美子　北大理植・札幌市南 26 条西 8丁目

牧野利一　札幌市南1条西 17 丁目　札幌医大微生物

増淵法之　北大理植・札幌市北8条西5丁目

松浦一　北大理植・札幌市北 1 条西 26丁目

松川昌弘　北海道虻田郡狩太町　狩太中学

三上日出夫　札幌市南 18 条西6丁目　札幌南高校・同市外中ノ島二区 139

三角　享　北大農植

向川信一　北大理植

宗像和彦　函館市八幡町　北海道学芸大学函館分校生物学教室

守谷　円　北大理植・札幌市北 20 条東 1丁目

山田幸男　北大理植・札幌市南 11 条西 13丁目

横山竜夫　北大農植・札幌市南2条西 12丁目　山田方

由崎俊道　札幌市南 22 条西 12 丁目　北海道学芸大札幌分校・同市南 4 条西 12 丁目　三条寮

吉村ふじ　北大農植・札幌市北 14 条西 2丁目　染野方

米田一郎　北海道学芸大理植・北海道常呂郡端野村字二区

和気和民　北大理植・同市北 18 条西 15丁目

渡会龍彦　北大理植・札幌市南 13 条西 10丁目　山田方

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北大理　札幌市北8条

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2. 東北支部

飯泉　茂　東北大理生物・仙台市南小泉門田北住宅7号

五十嵐俊雄　山形県新庄市十日町 106

石戸谷嘉夫　東北大理生物

伊倉伊三美　山形市六日町　山形大教育生物・同市十日町 441

伊藤直道　宮城県栗原郡築館町　県立築館高校

伊藤　弘　秋田県立花輪高校・秋田県鹿角郡花輪町新田町

岩田元信　盛岡市上田岩手大学芸生物・同大白蘭寮

岩崎俊郎　水沢科学博物館・岩手県水沢市福原 16

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木村有香　東北大理生物

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国谷雄三郎　群馬県館林市新宿 146

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黒木 [illegible]　塩釜市東塩釜東北海区水産研究所•同市小松崎 61
小林 [illegible]　福島市　福島大学芸生物
小[illegible]　岩手県[illegible]部中学
小山 [illegible]　秋田県立能代高校•秋田県能代市大町 51
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[illegible]　[illegible]
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[illegible]貞[illegible]　[illegible]
[illegible]　岩手県一関市山目沢内
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[illegible]野[illegible]　仙台市鈎取東台 38
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中条 [illegible]　仙台市東 3 丁目 62　科学館レージャーセンター•サイエンス•ルーム•同市荒巻住宅東 54 号
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[illegible]和　青森県下北郡川内町　戸沢中学校•同県同郡大湊町川守 16
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[illegible]之助　山形市小白川町　山形大文理
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[illegible]本広静　盛岡市内丸下ノ橋際 49
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吉岡邦[illegible]　福島市浜田町 福島大学芸生物•同市浜田町 84
和田俊司　東北大理生物

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伊東信吾　世田ケ谷区世田ケ谷 東京農大・豊島区雑司ケ谷 7-1110

伊藤武　品川区豊町 3-350

*伊藤洋　東京教大理植・文京区茗荷谷町 56

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井上浩　東京教大理植

井上行雄　世田ケ谷区世田ケ谷 3-2457

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昆野昭晨　北区西ケ原　農技研遺伝生理部
佐伯敏郎　東大理植
酒井文三　都立大理生物
酒井芳男　栃木県高久中学校・栃木県那須郡那須町高久橋本町　鈴木トシ方
坂本充　都立大理生物
坂本寧男　三島市谷田　国立遺伝研
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桜井廉　世田ケ谷区玉川上野毛町 115-1
佐々木一郎　大田区調布鵜ノ木町 231-10
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佐宗守　神奈川県足柄上郡中井村立中井中学・小田原市曾我源 518
佐竹義輔　台東区上野公園　国立科学博物館
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佐藤茂　新潟県中蒲原郡村松町乙 703
佐藤七郎　東大理植・文京区駒込林町 154
佐藤重平　東大教養生物
佐藤潤平　東京都北多摩郡清瀬村下清戸 1009
佐藤正一　東大理植・東京都北多摩郡東村山町久米川 113
佐藤昭二　世田ケ谷区池尻町　東京教大農
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志村令郎　山梨県大月市大月
下河原五郎　世田ケ谷区玉川尾山町 176　堀内方
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下間実　横浜市南区六つ川町大丸 1246 木原生物研
常谷幸雄　東京農大世田ケ区舟橋町 80
代谷次夫　東大理植・港区芝白金三光町 256
代谷康　東邦大学理学部・港区芝白金三光町 256
神宮寺誠　山梨県立甲府第一高校・甲府市塩部町 171
○神保一輔　新潟市二葉町 1-5214
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鈴木静夫　東京教育大理植・板橋区常盤台町 411
鈴木晋一　千葉県印旛郡白井村白井 400
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新関宏夫　平塚市　農技研

新津恒良　東大理植・目黒区下目黒 3 526

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西村光雄　東大理生化　(滞米中)

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塙順　都立大理生物・渋谷区幡ケ谷笹塚町 1362

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原田一　世田ケ谷区三宿町　昭和女子大

○久内清孝　大田区調布鵜ノ木町 231

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平井信二　東大農木材材料

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盛永俊太郎	北区西ケ原 農技研
守屋忠之	埼玉県秩父市 尾田蒔中学・同市 1913-1
門司正三	東大理植・千代田区富士見町 2-6-3
薬師寺英次郎	千葉県習志野市東邦大理生物・墨田区柳原町 7-2
◎保井コノ	お茶の水大理生物・文京区東片町 148
安田斉	松本市県町 信州大文理生物
安村明	北区稲付西町 3-29
矢野孝二	高田局区内 新潟大高田分校

山内　文	新宿区百人町　資源研・同区戸塚町 2-26
山浦　篤	埼玉大・板橋区板橋町 6-3440
八巻敏雄	東大教養生物
山本雅子	農技研
山岸高旺	東京教大理植
山崎　敬	東大理植・中野区鷺ノ宮 5-296-1　鷺ノ宮住宅
山崎照俊	立教大理生物物理
山崎典子	世田ケ谷区世田ケ谷 2-1169
山崎義人	平塚市　農技研
山田謙二	長野県中野市大字笠原
山田　保	千葉県印旛郡四街道町　千葉大教育・千葉県君津郡中郷村牛袋 183
山田義男	前橋市清王寺町　群馬大学芸生物
山本　茂	東京教大理植・新宿区西大久保 3-118
山本　徳	北多摩郡清瀬町上清戸 595-1
*湯浅　明	東大教養生物
横田菊江	栃木県足利市　県立足利女子高校
吉川　治	千葉市　千葉大文理生物
吉田精一	東大理植・文京区林町 92
吉田幸弘	都立大理生物・北区西ケ原4-36
*吉田吉男	新潟市　新潟大理生物
米田芳秋	東大理植
与安康代	横浜市神奈川区沢渡 18　神奈川学園高校
若林　裕	千葉県佐倉市鏑木　県立佐倉第二高校・同県山武郡松尾町松尾 34
和田善三	長野市箱清水県市長野西高校・同市西之門 929
和田文吾	東大理植・渋谷区神山町 6
渡辺　篤	世田ケ谷区世田ケ谷 3-2093
渡辺清彦	千葉市小仲台町　千葉大文理生物・同市松波町 97　県営アパート 101 号
渡辺成美	千葉市市場町　千葉大教育
渡辺良象	荒川区立第四日暮里小学校・豊島区池袋 3-1604
渡部一郎	千葉県東葛飾郡我孫子町妻子原 1634
亘理俊次	東大理植・市川市八幡町 1-689

（東大理　文京区本富士町，東大農　文京区向丘弥生町，東大教養　目黒区駒場町，東京教大理　文京区大塚窪町，都立大理　世田ケ谷区深沢町，お茶の水大理　文京区大塚町，横浜国立大学芸　鎌倉市雪ノ下，横浜市立大　横浜市金沢区六浦町，茨城大文理　水戸市渡里町，信州大文理　松本市県町，埼玉大文理　浦和市常盤町）

4. 北陸支部

旭　憲義	福井県大野郡鹿谷村西遅羽口
天羽良司	金沢大教育
有馬忠雄	金沢大理植
*今堀宏三	金沢大理植
梅鉢百子	金沢市横山町 1-41　岡田方
大田　弘	富山県下新川郡入善町椚山新
小野寺正二	福井大学芸・福井市春日泉町 1 号
蟹本信雄	福井市牧ノ島　藤島高校
香室昭園	福井大学芸
河合　功	金沢大理植
小林貞作	富山大文理生物
小牧　旌	石川県七尾市小島町 1
斎藤寛昭	福井県鯖江市中野町 16-2
斎藤佐一	福井県鯖江市川島町 34-14
堺　恵美	富山大文理・富山県魚津市大町 134
里見信生	金沢大理植
*柴田万年	富山大文理・富山市蓮町 22　大学官舎
島田郁雄	富山県福野町　県立福野高校
下道治一	福井県今立郡池田村寺谷
代崎良丸	小松市立犬丸小学校・石川県小松市大川町 178
進野久五郎	富山市東中野 125
*鈴木米二	富山大文理生物・富山市西宮28　桝田方
瀬嵐哲夫	金沢大教育生物
高桑　昇	富山県東砺波郡戸出町大清水
高野喜八郎	富山県射水郡小杉町　県立小杉高校・高岡市宮脇町 919
高橋節也	新潟県高田市南城町 2-8-5　県立高田高校
竹内　繁	福井県坂井郡長畝村篠岡 1618
竹内民男	福井市加茂河原町 32-8
谷口博之	金沢大理植
玉井直人	金沢大理植
津田道夫	金沢大理植
寺下友三郎	松波中学・石川県珠洲郡松波町秋吉

[illegible]
中□□七郎　富山大薬
長基健治　石川県鶴来町
西田晃二郎　金沢大理植
西村信義　石川県立小松高校・石川県能美郡大杉谷村瀬領
福島栄七　富山大学芸
古池博　金沢大理植・富山県西礪波郡福光町吉江中 1099
堀駿子　金沢市裏古寺町 14
[illegible]
*正宗厳敬　金沢大理植
柳沢勉　長岡市諏訪町 758　長岡高校
山岡正尾　富山県三成中学・同県中新川郡水橋町三郷中村 417
[illegible]
[illegible]
山田梯二　石川県加賀市大聖寺亀町 9

（金沢大理　金沢市仙石町，金沢大教育　金沢市弥生町，金沢大薬　金沢市大手町，福井大学芸　福井市牧島町，富山大文理　富山市蓮町）

5. 中部支部

天野一義　名古屋市千種区　城山中学
天野良之　静岡大文理生物・静岡市長谷町 13
飯島敬達　静岡県賀茂郡竹麻村湊 742
井沢□□　名大理生
石部修　津市大谷町　三重県立大水産
市村昭二　愛知県守山市大森金城台　金城学院大・生物物理研
伊藤道夫　名大理生物
福垣幸弘　三重県阿山郡阿山村千貝 914-1
井上俊　静岡大・農林学科　磐田市三宮枝村方
岩崎秀一　名大理生物
遠藤庄三　静岡大教育生物・静岡市東鷹匠町 12
及川公平　津市　三重大学芸生物
太田行人　名大理生物
太田敬久　名大理生物・愛知県守山市長栄 194
大原準之助　岡崎市桂町稲荷 22
大村敏朗　静岡市馬場町 6
岡田善敏　岐阜大・名古屋市千種区猪高町高針大廻間 4
岡本□嘉　甲府市　山梨大学学芸
*岡本尚　名大理生物
小沢立大　岐阜県武儀郡武芸村高野
加藤昌子　静岡大文理生物・静岡市追手町 76
加藤等次　愛知県宝飯郡一宮村金沢
加藤俊明　愛知県宝飯郡一宮村金沢
加藤幸雄　名大理生物
神谷平　愛知学芸大生物・愛知県安城市福釜新田 95
鴨川誠　名城大農学部　愛知県春日井町鷹来町 1
川松重信　三重県桑名郡長嶋村源部
熊谷三郎　岡崎市鴨田町郷前
熊沢正夫　名大教養生物・名古屋市瑞穂区弥富町月見ケ丘
倉内一二　愛知県立豊橋東高校・豊橋市牟呂町字若宮 109
倉地金光　名大教養生物
近藤静代　愛知県挙母市寺部中町 27
後藤正道　静岡県磐田郡佐久間町中部 436 の 2　佐久間高校
近藤武夫　静岡大浜松分校・浜松市広沢町 200
○斎藤賢道　名古屋市昭和区戸田町 3-19
斎藤全生　磐田市見付静岡大農・磐田市河原町 4055-3
佐藤徳次　愛知県立名古屋西高校・愛知県丹羽郡扶桑町柏森
沢井輝男　愛知学芸大名古屋分校生物・名古屋市昭和区北山町 2-1　北山荘 3-32
沢村保昌　津市　三重大学芸
志波秀雄　愛知県春日井市　名城大学農遺伝育種研
柴田和平　愛知県瀬戸市水無瀬町 37
*島村環　名大理生物
清水剛治　愛知県岡崎市丸山町美川中学・同市伊賀町字南郷中 24
志村義雄　静岡市大岩 343-8
須賀瑛文　愛知県丹羽郡丹陽中学・名古屋市昭和区元宮町 6-17
菅井道三　名大理生物
杉野武雄　岐阜県海津郡南濃町太田
杉本順一　静岡市八幡本町 5-9　杉本植物研究所

鈴木　昇　名古屋市瑞穂区高田町　愛知女子大学・名古屋市外鳴海町宿地 92　森川方
鈴木満帆　静岡大教養学部・掛川市塩町
瀬木紀男　津市大谷町　三重大水産・名古屋市昭和区広路町松風園 5
高尾昭夫　名大理生・津市阿漕町 2408
高木典雄　名大教養部生物
高野泰吉　名大農園芸　愛知県安城市新田町小山
高谷　修　岐阜県本巣郡糸貫村仏生寺・県立本巣高校
高橋千裕　名大教養部生物
高橋久之　静岡県藤枝市　藤枝西高校生物・同市千歳菓子久食品ＫＫ.
○高嶺　昇　名古屋市昭和区鶴羽町 3-8
滝崎吉雄　豊橋市瓦町南裏県営住宅Ａ16号
田中　潔　名大教養部生物
谷口森俊　三重県立大水産
寺尾恭平　三重県桑名高校・岡崎市明大寺町西郷中 39
土井田幸郎　名大理生物・岐阜県大垣市錦町 60
戸田英雄　浜松市広沢町 200　浜松市立高校・浜松市初生町 167
鳥居喜一　愛知県新城町西新町
中神時雄　豊橋市杉山町字殿村 21
中島光夫　名古屋市立向陽高校・愛知県守山市立大字上志段味 948
西井　久　三重県多気郡宮川村立荻原小学校栗谷分校
○野原茂六　浜松市上池川町 248
野村達郎　岐阜県鳥羽市上中島町 942
野呂　寿　三重県四日市市大字富田一色堺町浜
橋本竹二郎　名古屋市天白町八事裏山　名城大薬生薬
原田市太郎　名大理生物・名古屋市千種区田代町字楠 158　楠荘 129
日野精一　名大理生物
○日比野信一　名古屋市外天白村八事音聞山49
平井一男　名古屋市立大・名古屋市昭和区桜山町 2-44-2
平野　力　静岡県藤枝市五十海　県立藤枝西高校・静岡県志太郡広幡村構内
藤井良平　名大理生物
堀田康雄　名大理生物・名古屋市北区西志賀町 913
堀　武義　岐阜市　岐阜大学芸
堀米和雄　長野鉄道管理局中込車掌支区・長野県中野市西浦 1-767
前田英三　安城市名大農・岡崎市上六名町県営アパートＤ 18 号
水谷善弥　愛知県立瑞陵高校・名古屋市中村区大正町 2-50
南　喬二　浜松市高町 111　杉浦方
南川　幸　三重県三重郡菰野町　菰野高校・同町菰野 29021
村田新一　豊橋市松葉町 3-187
百瀬　昌　静岡県浜松市鴨江町 333
森　健志　名大理生物
森　隆也　岡崎市梅園町寺裏 6
谷田沢道彦　愛知県安城市　名大農肥料
矢頭献一　三重大農・津市広明町 85
山本幸男　名大理生物
横沢瑛二　名古屋市瑞穂区名古屋女学院高校・同区中山町 4 14
吉井義次　岐阜市外中町　岐阜大学本部
吉家やす子　名大理生
脇田晴美　名古屋市瑞穂区船原町 7-36
和田清美　静岡市北安東町 38

(名大理　名古屋市千種区，名大教養部　名古屋市瑞穂区，静岡大文理　静岡市大岩)

6. 近畿支部

赤井重恭　京大農植物病理
*芦田譲治　京大理植・京都市左京区下鴨北園町 106
阿部重美　阪大理生物
荒勝　豊　神戸市東灘区甲南大生物・神戸市東灘区本山町野寄
有賀裕勝　大阪市天王寺区南河堀町 43　大阪学芸大学生物学教室
有安　勉　京都市上京区紫野御所田町　京都学芸大学附属学園内
庵原　遜　大阪市立大理工
飯塚宗夫　京大食糧科学研究所応用遺伝研
生嶋　功　大阪市立大理工・大阪市生野区猪飼野中 1-23
池田勝彦　京都市左京区田中樋口町 38　藤田方
池田　元　神戸市東灘区御影町　神戸大理生物
石上　晃　兵庫県立洲本高校・洲本市筑地町　洲本高校職員寮
石田政弘　京大理植
依田恭二　大阪市大理工生物
依田静子　京大理植

伊藤五彦　京都市左京区下鴨北園町 37

稲垣幸弘　三重大学芸・三重県阿山郡阿山村大字千貝 914-1

*稲野藤一郎　大阪府箕面市大字小野原 1567

稲荷山資生　奈良市　奈良学芸大

井上[illegible]男　西宮市上甲東園　県立西宮高校

今村駿一郎　京大農応用植物・京都市左京区北白川上終町 28

厳佐耕三　[illegible]

*岩田五郎左衛門　兵庫県川辺郡西町加茂

岩田修[illegible]　神戸市東灘区御影町神戸大理生物・同市灘区将軍通 1-18　安田方

植田[illegible]二　奈良女子大理植・奈良市[illegible]町

上野[illegible]　[illegible]

梅崎[illegible]勇　舞鶴市長浜京大農水産・福井県大飯郡加斗村飯盛

内貴[illegible]　岐阜市長良岐阜大学学芸学部生物学教室

宇山晴子　大阪府松原市阿保町 166

浦山隆[illegible]　京大農応用植物・京都市左京区田中東飛鳥井町 10

大浦五郎兵衛　大阪女子大・大阪市天王寺区茶臼山町 40

大西健之　兵庫県加西郡多加野村馬渡合 377

岡本省吾　京大農林・京都市[illegible]宅町 72

岡本嘉　和歌山市西浜　和歌山大学学芸学部

小川[illegible]　[illegible]

奥田九郎　京都[illegible]大分校生物

奥貫一男　[illegible]

奥野春雄　京都工芸繊維大繊維植・京都市右京区花園円成寺町 12

奥村二郎　大阪府[illegible]高校・三島郡[illegible]町

小倉敏夫　京都市東山区林下町　華頂女子高校

小関治男　京大農遺伝

小辻望　阪大理生

梶良美　神戸市兵庫区有野町上向山

柏田豊　兵庫県城崎郡日高町兵庫蚕業試験場

勝見允行　京大理植

加藤一男　京大理植・京都市左京区浄土寺石橋町 81

加藤次郎　京大理植

神谷宣郎　阪大理生物・芦屋市三条町 81

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[illegible]　和歌山大学芸生物

[illegible]　大阪市立大理工・大阪市住吉区御崎町市営住宅 417 号

菊地[illegible]　京大理植

[illegible]　和歌山市和歌浦 1-142

[illegible]　阪大理生物

[illegible]　滋賀県甲賀郡水口町水口2207 2

[illegible]　京大農遺伝

[illegible]　京大理植

[illegible]　神戸市長田区菅原通 6-2　第三平和荘 222

[illegible]　阪大・京都市左京区銀閣寺町65

[illegible]　大阪市立大理工・大阪市東住吉区西今川町 6-29

[illegible]　神戸市東灘区神戸大教育・兵庫県有馬郡三田町貴志 815

[illegible]　京大理植・京都市北区出雲路神楽町

[illegible]　神戸市神戸大・兵庫県宝塚市仁川辰 1462

[illegible]太郎　京大理植・京都市左京区下鴨中川原町 96

工藤[illegible]夫　兵庫県宝塚市小林・小林聖心女子高校

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[illegible]衛[illegible]也　大阪市立大理工生物

小室英夫　京都女子大・京都市上京区寺町通鞍馬口下ル新御霊口町 285

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斎藤竜雄　大阪市[illegible]　府立高津高校

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佐藤一郎　奈良学芸大生物

沢　　　孝　阪大南校生物・大阪市東住吉区平野西ノ町 171　奥野方

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信　夫　隆　治　大阪市東住吉区平野　大阪学芸大分校・堺市浜寺元町 6-911

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杉　山　弘　幸　京都市東山区山科御陵中内町京都薬大・京都市左京区北白川山ノ元町 16

鈴　鹿　　紀　京都市東山区大宅坂辻町　日本新薬山科薬用植物研究所

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高　木　虎　雄　京都府立園部高校

高　嶋　弘　子　神戸市生田区下山手通 7-96 親和女子高校・竜野市竜野町竜野 40-4

高　田　　俊　神戸市須磨区大平町 2-107　山口方

高　田　　允　阪大理生物　神谷研

高　田　英　夫　大阪市立大理工生物

鷹　取　晟　二　豊中市柴原　阪大北校生物

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多　田　一　郎　大阪市港区三条通 4　大阪税関・大阪市阿倍野区万代西 1-4　税関寮

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田　中　美智子　滋賀県大津市膳所中庄殿町 346

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田　村　道　夫　大阪市阿倍野区王子町 3　阪大南校生物

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辻　　英　男　兵庫県篠山局区内　兵庫農大生物

坪　　田　宏　神戸大文理生物

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内　藤　良　子　大阪府布施市小若江近畿大通信教育部・大阪市生野区舎利寺町 1 68

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*中　沢　亮　治　尼崎市塚本元町 5

中　島　岩　通　阪大理・附属蛋白質研

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[illegible]田　稔　京大農応用植物・京都市左京区永観堂町 33

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森　[illegible]祐[illegible]　阪大理生物

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山岸秀夫　京大理・植物細胞研究室

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吉原朝子　奈良女子大理植・京都市中京区高倉夷川上ル

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渡辺弘三　滋賀県甲賀郡甲南町深川

（京大理　京都市左京区北白川，京大農　京都市左京区北白川，京都工藝繊維大　京都市北区大将軍坂田町，阪大理　大阪市北区，大阪市立大理工　大阪市北区南扇町，奈良女子大　奈良市北魚屋西町，神戸大理　神戸市東灘区御影町，和歌山大学藝　和歌山市真砂町）

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宇野確雄	岡山市清心女子大・倉敷市浅原 836
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河合　晃	鳥取市吉方 320　全国椎茸普及会本部
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斎藤雄一	鳥取市　鳥取大農
○坂村　徹	広島市古田町古江 1370
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佐々木好之	広島大理植
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竹本貞一郎　岡山大教育生物・岡山市門田 283
太刀掛優　呉市二河通　市立二河小学校
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Mitochondria Diminution in Hypertrophied *Equisetum* Spores

by Singo NAKAZAWA*

中沢信午*: 巨大化したスギナ胞子におけるミトコンドリアの減少

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Equisetum spores never form a rhizoid but become hypertrophied when they are cultured in a medium containing colchicine at a concentration of 0.01 per cent. But if the hypertrophied spores are transferred to a normal culture, they begin to differentiate a rhizoid.[1,2,3] A Similar phenomenon is reported also in *Funaria*.[4] On the other hand, it is well known that the morphogenetic pattern is characterized by mitochondrial enzymes[5]. For instance, in sea urchin eggs, the distribution density of mitochondria is larger in the animal pole region than in the vegetal region. Therefore, vegetalization occurs when the egg is reared in a medium containing an agent such as LiCl which inhibits the synthesis of mitochondrial enzymes[6),7),8)]. Herein, a research was undertaken to make clear a question as to how mitochondria behaved with occurrence of hypertrophy and with their regain of potency to form rhizoid.

The experiments were carried out extending over April to May, 1958, on fresh spores of *Equisetum arvense* collected in Yamagata. The spores were reared in the culture medium contained in Petri dishes, 40 mm. in diameter and 15 mm. in depth, sown at a density of 400 to 500 individuals for one dish. The dish was covered, and incubated at $20 \pm 1°$. The wall of the incubator was made of colorless vinylon, so that the spores were kept under a diffused white light. Diluted Knop's solution, 1/5 in density, was applied for the control culture medium. For inducing hypertrophy, colchicine was added to the control medium. Density of colchicine was adjusted to 0.10, 0.05, 0.02, and 0.01 per cent. The hypertrophied spores were transferred to the control medium after being rinsed with tap water to let them regain the ability of forming rhizoid. For proving mitochondria, spores were centrifuged at 10,000 times gravity for 20 minutes, and stained with 0.001 per cent Janus green B.

As is shown in Table 1, hypertrophy occurred with colchicine in a high ratio. The hypertrophy is classified into two different types. One is the polar hypertrophy which occurred in media containing 0.01 to 0.05 per cent of the agent. In these media the spores grew up without cleavage to ten or more times the control in volume, and became elongated to an ovate or a spatulate form pointed at one end (Fig. 1E). Chloroplasts are more densely distributed in the blunter half. The other type is the apolar hypertrophy occurring in a medium containing 0.1 per cent colchicine.

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Table 1. Induction of hypertrophy in media containing colchicine.

Colchicine density (%)	Normal germination (%)	Hypertrophy (%)	Dead (%)	Spores observed (%)
0.10	0	43	57	101
0.05	0	45	55	113
0.02	0	99	1	120
0.01	85	14	1	109
Control	99	0	1	100

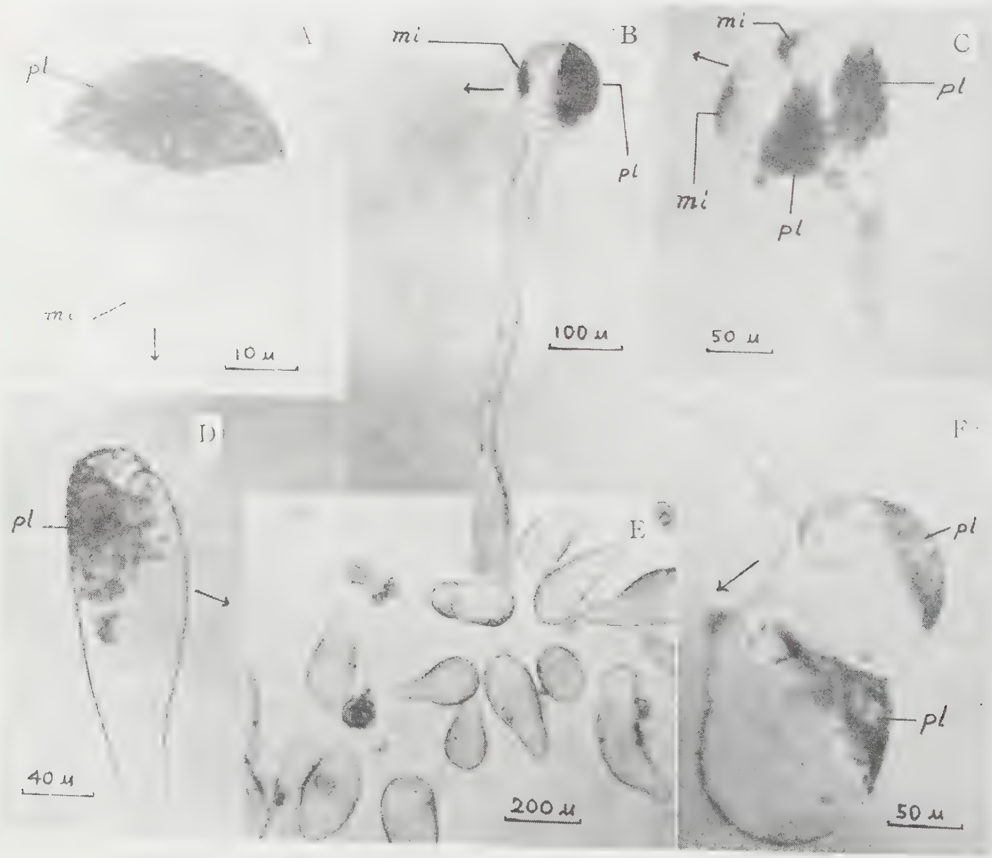

Fig. 1. *Equisetum* spores. A, normal spore centrifuged after expansion taking water in the control medium; B, the same at a stage forming rhizoid; C, the same at a later stage having two protonema cells; D, polar-hypertrophied spore centrifuged; E, a group of the same before centrifugation; F, hypertrophied spore centrifuged at a later stage forming rhizoid being transferred to the control medium. mi, mitochondria; pl, chloroplasts; arrow indicates centrifugal direction.

In this case, the spore became a giant globe without elongation having diameter four to five times larger than the control. The nucleus is situated in the center surrounded by chloroplasts compactly. Regardless of the type they belong to, the hypertrophied spores cannot form rhizoid so far as their medium is not altered. But if the polar-hypertrophied spores are transferred to the control medium, segmentation takes place and the pointed end cuts out a protuberance which is elongated to become a rhizoid (Fig. 1F). The apolar-hypertrophied spores neither can form rhizoid nor can be segmented even if they are transferred to the control medium. When the fresh spore is centrifuged at 10,000 times gravity for 20 minutes, the intracellular materials are clearly stratified into the oil cap at the centripetal end, layer of chloroplasts with nucleus in the next, the hyaline zone, and mitochondria gathered at the centrifugal end. The mitochondria are stained blue with Janus green B. When the fresh spores, about 40 μ in diameter, are sown in a culture medium, they expand after several hours to about 60 μ in diameter absorbing water. If they are centrifuged at this time, the oil cap does not appear so that the chloroplasts are gathered at the centripetal end, while mitochondria are also stratified at the centrifugal end (Fig. 1A). When a spore germinated normally is centrifuged, chloroplasts with nucleus are stratified at the centripetal and mitochondria at the centrifugal end of the protonema cell (Fig. 1B, C), while stratification is not always clear in rhizoid. Nevertheless, it is obvious that the rhizoid contains many mitochondria, which can be detected with Janus green B. Very few or no mitochondria are stratified with centrifuging, nor can they be detected by use of Janus green B in hypertrohied spores. As aforesaid, the polar-hypertrophied spores germinate and form rhizoid when they are transferred to the control medium. Testing them at this stage by means of centrifuging and staining with Janus green B, it became clear that mitochondria were also diminished in the protonema (Fig. 1F), but were abundantly contained in the rhizoid.

Results above indicate that the presence of mitochondria is in some way connected with the rhizoid differentiation. That is, while a number of mitochondria are contained in the normal spore, they are diminished in the hypertrophied spore which does not form rhizoid so far as its culture medium is not altered. If the hypertrophied spore is transferred to a colchicine-free medium, it forms rhizoid. At this time, an abundance of mitochondria reappear in the rhizoid while they are yet diminished in the protonema cell. But it is a further question that whether the reappearance of mitochondria is a cause or a result of the rhizoid differentiation.

Summary

(1) Spores of *Equisetum arvense* were reared in media containing colchicine. As a result, hypertrophy was induced in a high ratio. The effective range of colchicine density was 0.01 to 0.05 per cent.

(2) The normal spore contains many mitochondria, and they are stratified at

the centrifugal end by means of centrifugation at 10.000 times gravity. Upon germination, they are also contained both in rhizoid and in protonema.

(3) Mitochondria are diminished in number or are not to be perceived in the hypertrophied spore which is incapable of forming rhizoid so far as its medium is not altered. Rhizoid is differentiated when the hypertrophied spore is transferred to a colchicine-free medium. On this occasion, mitochondria reappear in that rhizoid as many as those in the normal germination, while they are yet diminished in the protonema.

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摘　要

(1) スギナ (*Equisetum arvense*) の胞子は多くのミトコンドリアを有し，それらは 10,000 g の遠心力で遠心端に集められる (図 1 A). 正常の発芽にあたり，ミトコンドリアは仮根細胞にも原糸体細胞にも含まれている (図 1 B, C).

(2) コルキシンを含むクノープ液で胞子を培養し，一側に尖つた (卵形) 有極的な巨大化をおこさせることができた(図 1 E). この巨大化に有効なコルキシン濃度は 0.01〜0.05% であつた.

(3) 巨大化した胞子は培養液をとりかえないかぎり仮根を形成しない。他方において，巨大化した胞子にはミトコンドリアがまったくないか，またはほとんど含まれていない (図 1 D). 巨大化した胞子をコルキシンを含まないクノープ液にうつすと仮根形成がおこり，また原糸体が発生する。このとき，原糸体細胞にはやはりミトコンドリアが欠乏しているが (図 1 F), 形成された仮根内には正常の発芽におけると同じく多数のミトコンドリアがふたたび出現する。

On the Establishment of the Vertical Distribution of Photosynthetic System in a Plant Community*

by Toshiro SAEKI** and Sumio KUROIWA**

佐伯敏郎**・黒岩澄雄**：植物群落における光合成系の垂直分布の成り立ち

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Since the introduction of "stratifying clip method" by Monsi and Saeki[1], some of the characteristic features of productive structure in plant communities have been cleared up. One of the most attracting among them is the extensive similarity in the characteristic form of vertical distribution of leaves (Fig. 1) in the land plant community[2],[3],[4],[5],[6],[7],[8],[9]. Monsi and Saeki[1] indicated that such vertical distribution-structure of leaves of *Sanguisorba tennuifolia* developed along the same line as its own vertical productivity gradient. Furthermore, it should be emphasized that phytoplankton communities in waters often take similar distribution profile to that in land plant communities. The establishment of such vertical distribution-structure of phytoplankton was interpreted by Hogetsu and Ichimura through the calculation of the production and reproduction of the phytoplankton[10].

Those works may admit a basic assumption in this report that the vertical distribution-structure of leaves or phytoplankton is determined by the productivity. Based upon this assumption and the light-leaf amount relation[1], the authors present theoretical formulae as regards vertical distribution-structure of leaves in plant community, and they discuss the appliance of the formulae not only to the land plant community but also to the phytoplankton community, on the basis of a model experiment. Some ecological meaning of those formulae will also be discussed.

Theoretical formulation

As clearly shown with the "stratifying clip method"[1], light intensity diminishes markedly within a plant community. The decreased light must physiologically bring about the depression of photosythetic performance of the leaves. This depressed photosynthesis will naturally cause the delay of development of photosynthetic system, because new leaves will be produced by the raw material assimilated through photosynthesis. The same explanation is applicable to the phytoplankton community in

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stagnation period in lakes[10),11)]. So the authors assumed that the leaf (or phytoplankton) density $\Gamma(x)$ at a depth x (measured from the top of the plant community) is proportional to the photosynthetic rate q, which is confined by the light intensity I prevailing at x. That is,

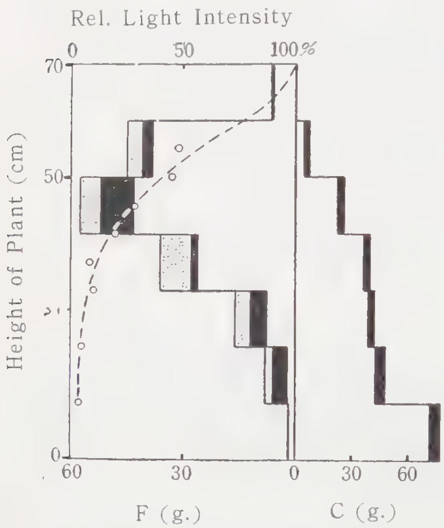


Fig. 1. The productive structure of a *Sasa nipponica*-community, demonstrated with the stratifying clip method. Photosynthetic system (F) consists of only the laminae; non-photosynthetic system (C) of the leaf sheaths, stems, etc. in fresh weight in a 50 cm. square. Black polygons mean the system of other ədscies, and the dotted the yellowing leaves.

$$\Gamma(x)=cq \tag{1}$$

where c is a constant. Hourly or daily light-photosynthesis curve of leaves or phytoplankters is generally represented with the following equation[12)],

$$q=\frac{bI}{1+aI}-r \tag{2}$$

where a and b are constants, and r is respiration rate. Provided F is the total area of leaves per unit ground area distributing from the top to a depth x in the plant community, i. e.,

$$F=\int_0^x \Gamma(x)\,dx \tag{3}$$

The light intensity I received by leaves at x is determined by means of the equation,[1)]

$$I=KI_0\exp[-KF]=KI_0\exp[-K\int_0^x\Gamma(x)dx] \tag{4}$$

where transmissibility of leaves is neglected as being small. Extinction coefficient K is a constant characteristic of a plant community. Equations (3) and (4) are also applicable (when F is replaced by the quantity of phytoplankton itself) to the relatively shallow waters where no particles except phytoplankton are intercepting light.

From Equations (1), (2) and (4), we can derive the following Equations (5) and (6) which indicate the relations between I and x, and between $\Gamma(x)$ and x, respectively.

$$\frac{1}{aI_K}\ln\left(\frac{I}{I-I_C}\right)+\ln\frac{1}{a(I-I_C)}=Kcq_0x+\frac{1}{aI_C}\ln\left(\frac{I_0}{I_0-I_C}\right)+\ln\frac{1}{a(I_0-I_C)} \tag{5}$$

$$\frac{q_0}{r}\ln\left(\frac{q_0}{q_0+r}\right)\left(1+\frac{cr}{\Gamma(x)}\right)+\ln\left(\frac{q_0}{q_0+r}\right)\left(\frac{cq_0}{\Gamma(x)}-1\right)=Kcq_0x+\frac{1}{aI_C}\ln\left(\frac{I_0}{I_0-I_C}\right)+\ln\frac{1}{a(I_0-I_C)} \qquad (6)$$

where I_C is the light intensity at the compensation point of leaves, and q_0 is the maximal net photosynthetic rate when I is infinite. If the respiration is neglected because of its relatively small role in the productivity—as well known, the respiration rate is generally only one tenth of the photosynthetic rate,—Equations (5) and (6) are simplified as follows;

$$\frac{1}{aI}+\ln\frac{1}{aI}=\frac{b'}{a}(x-x_0) \qquad (7)$$

$$\left(\frac{\Gamma_0(x)}{\Gamma(x)}-1\right)+\ln\left(\frac{\Gamma_0(x)}{\Gamma(x)}-1\right)=\frac{b'}{a}(x-x_0) \qquad (8)$$

where $b'=Kbc$, $x_0=-\frac{a}{b'}\left(\frac{1}{aI_0}+\ln\frac{1}{aI_0}\right)$ and $\Gamma(x)\xrightarrow[I_0\to\infty]{}\Gamma_0(x)=\frac{b'}{a}$.

As illustrated in Fig. 2, $\Gamma(x)$ is shown in a curve continuously decreasing and approaching zero with increasing depth. The point of inflexion of the curve is 2/3 $\Gamma_0(x)$. This curve well corresponds to the natural vertical distribution of phytoplankton in lakes, especially in eutrophic ones, in a stagnation period.

In waters, occasionally, a large quantity of abiotic seston makes the application of the above assumption difficult through strong interception of light, hence it is necessary to have some revision of the theoretical equations. However, there may be no difference in the shape between the curves calculated with the revised equation[13] and those calculated with Equation (6) or (8).

Concerning the shape at the uppermost part the structure of the land plant community is different from the theoretical curve. Later will be mentioned in this respect.

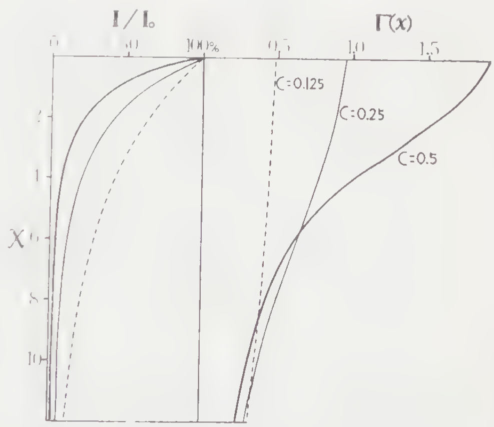


Fig. 2. The relation of relative light intensity I/I_0 and the density of photosynthetic system $\Gamma(x)$ to the depth x, based upon theoretical Equations (7) and (8), in which $a=b=2$, $K=2$ and $I_0=5$. c denotes the constant of proportionality in Equation (1).

Experimental evidence

For the purpose of further evidence of adequacy of Equations (7) and (8) the authors carried out model experiments. The experiments were based on examining how the initial homogeneous distribution of photosynthetic system changes during the successive growth under the condition imitating natural plant community. Seven or twelve shallow glass dishes in the same size (diameter: 17 cm., depth: 2.5 cm.) were piled up, each of which contained an identical quantity of *Chlorella ellipsoidea* suspended in 200 ml. culture solution. The side wall of the piled dishes was kept light-proof with a sheet of black paper, so that light could penetrate only through the top, and it was decreased gradually towards the base mainly by shading effect of the suspended *Chlorella*. These experiments were undertaken in autumn (1954) and in late winter (1955) in a green house.

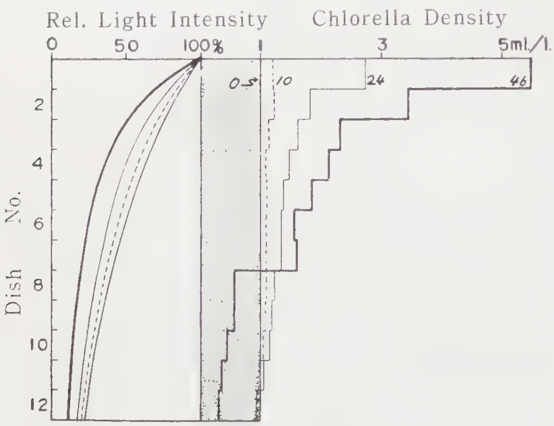


Fig. 3. Development of vertical distribution of *Chlorella ellipsoidea* and relative light intensity in piled dishes. Initial concentration of *Chlorella* suspension is 1 mg./l.. Samples were taken 10, 24, and 46 days after inoculation.

An example of the development in the vertical structure obtained from the experiments is illustrated in Fig. 3, and the physiological characters of the algae at the latest stage are summarized in Table 1. It is obvious that these experimental curves accord with the theoretical ones in Fig. 2. It may also be noticed that the curve at the later stage corresponds to a curve obtained in the case of higher value of constant c.

Table 1. Physiological characters of *Chlorella ellipsoidea* cultivated within piled dishes for 45 days. Net photosynthesis (7000 lux, 20°) and respiration (18°) were measured by Winkler's method. p.c.v. stands for packed cell volume.

Petri dish No.	2	4	6	8	10	12
Net photosynthesis mg. O_2/hr. ml. (p.c.v.)	21.8	19.7	16.7	15.2	18.2	12.1
Respiration mg. O_2/hr. ml. (p.c.v.)	0.94	0.80	0.91	0.85	—	0.94
Chlorophyll content mg./ml.:(p.c.v.)	5.5	6.0	7.5	12.5	11.5	8.0

Further modification of the formula

In the real land plant community (cf. Fig. 1) the leaf density decreases not only

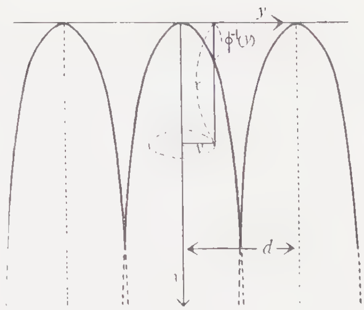


Fig. 4. Schematic diagram of a plant form.

in the lowermost part but also in the uppermost part. This decrease in leaf density in the uppermost part is able to be expressed by revision of the Equation (6) or (8), introducing an assumption that each individual plant in the community takes an apparent form as illustrated in Fig. 4, typical example of which is met with in forest trees. Namely, provided that the plant form is expressed with $\phi(x)$ (see Fig. 4) and the plants are distributed in a regular triangle disposition, $\Gamma(x)$ would be now revised as following $F(x)$.

$$\left.\begin{aligned}
&\text{When } x=0\sim\phi^{-1}\left(\tfrac{d}{2}\right)\\
&\quad F(x)=\frac{2}{\sqrt{3}d^2}\int_0^{\phi(x)}2\pi y\Gamma\{x-\phi^{-1}(y)\}dy\\
&\text{and when } x=\phi^{-1}\left(\tfrac{d}{2}\right)\sim\infty\\
&\quad F(x)=\frac{2}{\sqrt{3}d^2}\int_0^{d/2}2\pi y\Gamma\{x-\phi^{-1}(y)\}dy
\end{aligned}\right\} \quad (9)$$

where d denotes the interval between two individuals.

Now, if $\phi(x)$ is approximated with the equation,

$$\phi(x)=\left(\frac{m^2x}{n^2+x}\right)^{\frac{1}{2}}, \qquad (m \text{ and } n \text{ are constants})$$

Mean value of $\phi^{-1}(y)$ is enough small in comparison with x, and Equations (9) are rewritten as follows.

$$\left.\begin{aligned}
&\text{When } x=0\sim\frac{n^2d^2}{4m^2-d^2}\\
&\quad F(x)=\frac{2\pi}{\sqrt{3}d^2}\left\{\frac{m^2x}{n^2+x}\Gamma(x)+m^2n^2\left(\ln\frac{n^2}{n^2+x}+\frac{x}{n^2+x}\right)\Gamma'(x)\right\}\\
&\text{and when } x=\frac{n^2d^2}{4m^2-d^2}\sim\infty\\
&\quad F(x)=\frac{2\pi}{\sqrt{3}d^2}\left\{d^2/4\,\Gamma(x)-n^2\left(m^2\ln\frac{m^2}{m^2-d^2/4}-d^2/4\right)\Gamma'(x)\right\}
\end{aligned}\right\} \quad (10)$$

Curve $F(x)$ is characteristic in the sharpness of the top part, as shown in Fig. 5, therefore, just the typical distribution-structure of the land plant community was obtained here.

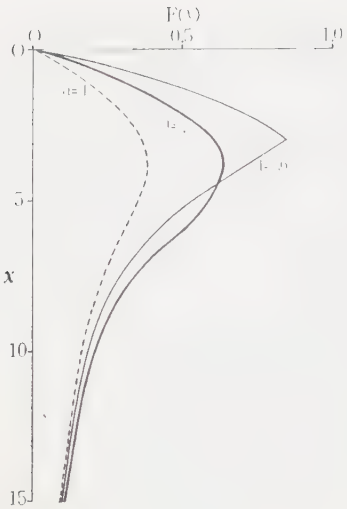


Fig. 5. Vertical density-distribution of photosynthetic system in land plant community calculated with theoretical Equation (10), in which $a=b=2$, $c=1$, $K=1$ and $I_0=20$.

Furthermore, we can obtain curves of various shapes according to the difference of population density, as the decrease of the interval d implies the increase of population density. Such calculation shows that the greater the population density, the more the leaves are compressed at the upper part, as seen in Fig. 5. This phenomenon has been realized in an experimental cultivation of *Fagopyrum esculentum* with three grades of density (Kuroiwa and Monsi)[6]

Also the phytoplankton distribution in lakes often shows the similar curves of decreasing density in the uppermost part,[11] to the typical structure curves of land plant communities. For this distribution, however, the inhibited photosynthesis of phytoplankton by too strong light[15] seems to be responsible, so that another modification must be adopted here. Employing an equation which represents such light-photosynthesis curve characteristic of phytoplankton in stead of hyperbola in Equation (2), we can easily obtain a top-diminished distribution curve just concerned.

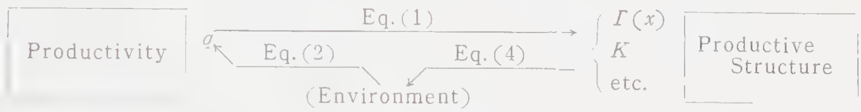
Ecological meaning of the equations

The theoretical equations have proved their soundness and the applicability to ecological situation as shown in the foregoing. It may have a great meaning to interpret clearly the close interrelationships between productive structure and productivity, and between 'action' and 'reaction' (after Clements) by means of these equations.

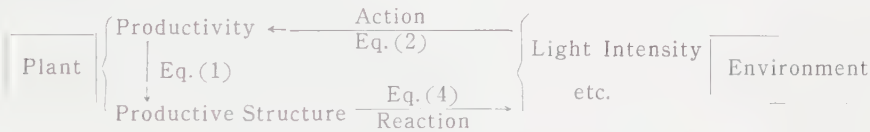
Productive structure will be defined as the structure which determines productivity. Equation (4) indicates that the vertical distribution-structure of leaves $\Gamma(x)$ as well as the extinction coefficient K determines the light intensity at x, and thus further determines productivity q according to Equation (2). Therefore, both the vertical distribution-structure of leaves and the extinction coefficient are the major factors determining productivity, and consequently they are the important constituent factors of productive structure.

On the other hand, effects of productivity upon productive structure are interpreted as follows. Equation (1) indicates that productivity q is a determining factor for the formation of vertical structure of leaves $\Gamma(x)$. The influence of productivity q seems not to be so direct upon the extinction coefficient K, as upon the vertical distribution-structure $\Gamma(x)$, and the extinction coefficient ordinarily appears to be independent of the productivity. Taking longer evolutional period into consideration,

however, it may be admitted that also the latter plays an important role in determining the direction of historical change of the former, because the characters of a plant species, including the extinction coefficient, may not be maintained without survival of the species, which can be supported only by the higher productivity under given environmental conditions in a long period.



The interrelationships between a plant or plant community and its environment, which are usually analysed as 'action' and 'reaction' after Clements, will be elucidated by means of this formulation more clearly. The environment acts on the plant and modifies the plant in its structure, and the plant reacts on its environment and changes more or less the environmental factors. Concerning production of matter, the plant or plant community can be divided into two opponent factors, i. e. productivity and productive structure.



The 'action' of the environmental factors, under mesophytic condition, first expresses itself as influence of light intensity on the productivity. Equation (2) indicates such relationship. The influences of the other environmental factors, such as temperature, water, carbon dioxide, nutrient salts, etc. express themselves in terms of the changes in the shape of light-photosynthesis curves[16),17)], that is, changes of values of the constants a, b and r in Equation (2). The productivity varied by the 'action' reveals itself in the development of the productive structure according to Equation (1), being influenced by the genetical characters of the species and by further 'action' of the environmental factors on the structural development process. The changed productive structure reacts on the environment, following Equation (4), in the light intensity in the plant community, and in the other factors such as temperature, humidity, carbon dioxide concentration, soil relationships, etc. Among them, the first and the third have been discussed in detail by Kuroiwa and Monsi[6)], and Midorikawa[7)].

Summary

1. Concerning the vertical light distribution and density-distribution of leaf or phytoplankton, theoretical equations have been formulated [Equations (5), (6) and their simplified forms, Equations (7), (8)], on an assumption that such density-distribution is formed proportionally to the productivity of leaves or phytoplankton.

2. Theoretical curves thus obtained are practically of the same type as the curves obtained in a phytoplankton community in stagnation period of waters and they also accord well with the characteristic of the lower part of leaf distribution in the land plant community.

3. In order experimentally to prove these theoretical equations, seven or twelve shallow glass dishes were piled up, each of which contained 200 ml. of *Chlorella ellipsoidea* suspension in the same concentration. The development of vertical *Chlorella* distribution (Fig. 3) accorded considerably well with that calculated with Equation (8) (Fig. 2).

4. By referring to the common growth form of land plants (Fig. 4), theoretical Equation (6) or (8) has been revised as Equation (10), which well expresses the characteristics of the vertical leaf distribution of land plant community (Fig. 5).

5. Interrelationships between productive structure and productivity of plant community, and between 'action' and 'reaction' were interpreted, based upon the theoretical equations.

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摘　　要

層別刈取法によってえられる陸上植物群落の葉の垂直分布は多くの群落で共通であり，また湖沼の植物プランクトンの停滞期における垂直分布も同様である。光合成系の垂直分布のこのような広い共通性を説明するために，次の仮定 ——群落内の任意の深さにおける光合成系の量がそこでの生産力に比例して形成される—— と，光と光合成系の既知の量的関係から，光と光合成系の垂直分布の形を数式化した (式(5), (6))。 呼吸は光合成にくらべて小さいので，これを省略すると一層簡単な式 (7) と (8) になる。これらの理論式からえられる光合式系の垂直分布の形 (Fig. 2) はしばしばあらわれる植物プランクトンの分布の形とよくにており，陸上群落でも葉の分布の下の部分をよく表現する。これの実験的証明をうるため，同形のシャーレを 7～12 段積み重ね，その側面を黒紙でおおい，光は最上段の上部から透入するだけとし，各シャーレには等量の *Chlorella ellipsoidea* を入れ，温室内で培養した。日時とともに各段のシャーレにおける生長量と光の分布に差があらわれ (Fig. 3)，それらは理論式から計算される垂直分布 (Fig. 2) とよく一致した。陸上植物群落の葉の垂直分布の最上部の形については理論式とそれと差があるが，われわれは高等植物特有の形態を考慮して，実際の分布形に一致する新しい理論式 (10) を導きだした (Fig. 5)。また群落密度の変化にともなって生ずる分布形の変化も同じ式から導きだした。以上のべた光合成系の垂直分布を中心とする生産構造と，生産量，および植物と環境の相互作用の意義を理論式を通じて明らかにした。

A Paper Chromatographic Survey of Gibberellins and Auxins in Immature Seeds of Leguminous Plants

by Yutaka MURAKAMI*

村上 浩*: マメ科植物の未熟な種子に含まれるジベレリンとオーキシンのペーパークロマトグラフィーによる調査

Received October 20, 1958

The gibberellins, which are metabolic produts of the fungus *Gibberella fujikuroi*, promote the growth of many plant species[1]. In particular they greatly stimulate the growth of the *Japonica* type of rice plants. Studies on the mode of action of gibberellins in cereal leaves led the writer to advance the fact that growth promoters distinct from auxins participate in the plant growth regulating system[7]. Recently extracts which possess similar activity to that of gibberellins have been obtained from the seed of several higher plants[2)3]. MacMillan and Suter[4] have isolated gibberellin A_1 from the seed of *Phaseolus multiflorus*.

This article reports the results of a paper chromatographic survey of gibberellins and auxins which are present in immature seeds of 15 species from different genera of Leguminosae.

Materials and Methods

The following species were examined: *Dolichos Lablab, Vigna sesquipedalis, Phaseolus angularis, Canavalia gladiata, Glycine Max, Pisum sativum, Arachis hypogaea, Robinia pseudo-Acacia, Wistaria floribunda, Medicaga sativa, Cytisus scoparius, Maackia amurensis, Sophora angustifolia, Cercis chinensis* and *Albizzia Julibrissin*.

The methods of plant extraction, chromatography and bioassay are essentially similar to those used in the previous paper[2].

Extraction: Immature seeds taken from pods were covered with ether and allowed to stand for 2 days at room temperature. The solution was then filtered and the residue was extracted again for further 2-3 hours. The combined extract was evaporated under reduced pressure and the residue was dissolved in a small volume of acetone for paper choromatographic studies.

Paper chromatography: Suitable quantities of the extracts to be examined were developed on paper chromtograms with the solvent mixture of isopropanol/water/ammonia (D=0.88) (10: 1: 1) at about 27° for 16 hours using the ascending technique. The solvent front travelled 32 cm. under these conditions. Indoleacetic acid (IAA) and,

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occasionally, gibberellin A mixture were run in parallel with plant extracts in order to determine their standard position. The paper was then dried and cut lengthwise into strips of 2 cm. in width. The strip was again cut into 2 cm. sections at right angles to the direction of the solvent flow. Each piece thus obtained was placed in beakers 3 cm. in diameter and 7 cm. in height and bioassayed. The fresh weight of seed material, from which each extract was obtained, is given in brackets with the name of the species in the legend of Figures 3 and 5. Colour tests for indole compounds were carried out on the strips with Ehrlich's reagent (2 g. p-dimethylaminobenzaldehyde in 20 ml. HCl+80 ml. ethanol).

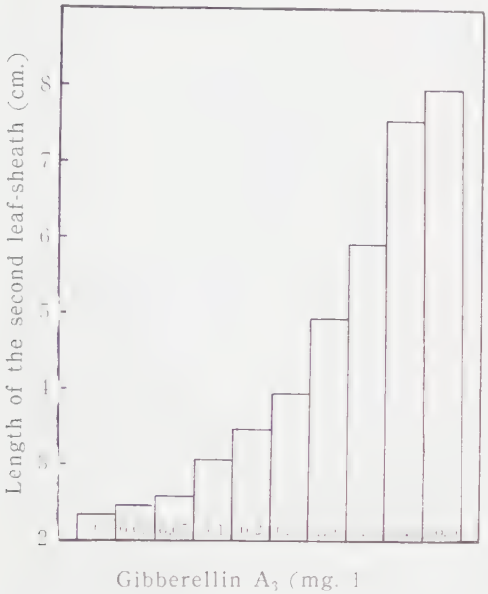

Fig. 1. Response of the second leaf-sheath of rice seedling to gibberellin A_3.

Bioassay: The gibberellin-like activity was measured by the rice seedling method[2], which is specific to gibberellins. In this method five seedlings, whose coleoptiles attained about 1 mm. were planted on each paper piece immersed in 1 ml. distilled water and allowed to grow under the ordinary daylight conditions at about 25 28°. They were supplied with 0.5 ml. water every day. The length of the second leaf-sheath was measured after 7 days. A result typical of the rice seedling method is given in Fig. 1. It showed that the lower limit of sensitivity is at concentration of 0.05 mg./l gibberellin A_3. IAA and kinetin were found to have no effect over the range 0.1 to 10 mg./l in this bioassay.

Auxins were measured by the straight-growth test using oat coleoptiles. The coleoptile sections 3.1 mm. in length were placed on each paper piece immersed in 0.5 ml. of distilled water. The beakers were then allowed to stand in the dark at 27° and the length was measured after 18 hours. The growth of *Avena* sections under these conditions is shown in Fig. 2. IAA at 0.01 mg./l caused a slightly lesser growth than water and the lower limit of sensitivity was at 0.1 mg/1 IAA. Their response to gibberellins is very small[5].

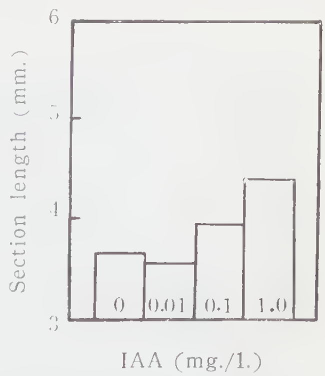

Fig. 2. Response of *Avena* coleoptile section to IAA.

Initial length of section was 3.1 mm.. Test solution was 0.5 ml..

Results and Discussion

The results of gibberellin-like activity are summarized in the form of histograms

showing the length of rice leaf-sheath on chromatograms. Although impurities in the extracts caused variations in both the Rf and the tailing of the active substances on chromatograms, the form of histograms may be classified into three types depending on the position of growth-promoting activity. *Arachis hypogaea* (Fig. 3-A), *Cytisus scoparius* (Fig. 3-C), and *Wistaria floribunda* (Fig. 3-I) are representative species of each type, respectively.

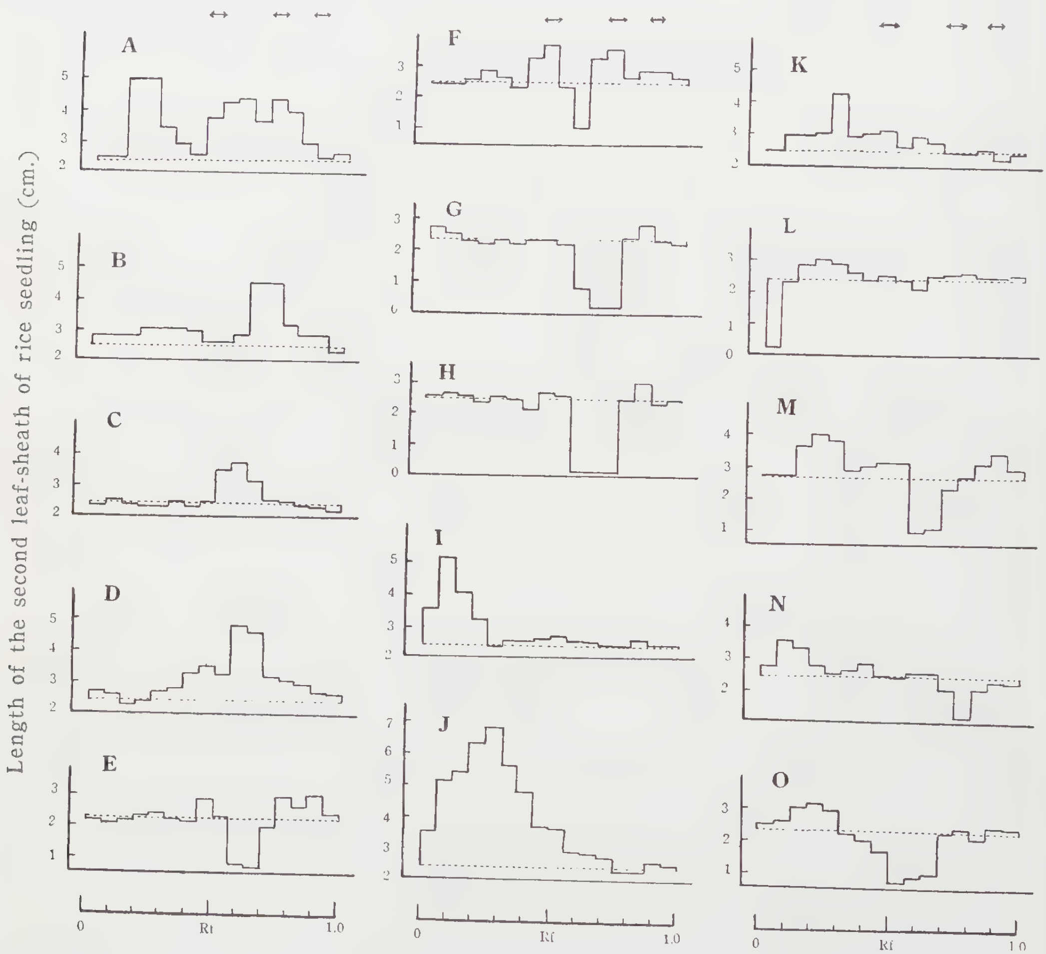

Fig. 3. Histograms showing gibberellin-like activity of ether extracts of immature seeds of Leguminosae after paper chromatographic development with ammoniacal isopropanol. Broken lines denote water controls. Arrows at the top of the histograms indicate positions of IAA (Rf=0.45), gibberellin A (Rf=0.75), and IAN (Rf=0.85), respectively.

A, *Arachis hypogaea* (14 g.); B, *Robinia pseudo-Acacia* (12 g.); C, *Cytisus scoparius* (10 g.); D, *Sophora angustifolia* (4.5 g.); E, *Phaseolus angularis* (24 g.): F, *Vigna sesquipedalis* (30 g.); G, *Glycine Max* (10.5 g.); H, *Dolichos Lablab* (28 g.), I, *Wistaria floribunda* (3.2 g.); J, *Maackia amurensis* (12 g.); K, *Albizzia Julibrissin* (30 g.); L, *Cercis chinensis* (8.4 g.); M, *Canavalia gladiata* (9 g.); N, *Pisum sativum* (8 g.); O, *Medicago sativa* (4.9 g.).

There were two growth-promoting zones on the chromatogram of the extract of immature seeds of *Arachis hypogaea*. One zone corresponded to the position of gibberellin A and the other promoting zone had an Rf value far less than that of gibberellin and lay near the starting line. The Rf value of the latter zone was of the same order as that of the growth-promoting zone of *Wistaria floribunda*. Similar two peaks of growth activity were also found with the extract of *Robinia pseudo-Acacia* (Fig. 3-B). However, the peak near the starting line of the chromatogram was much lower than that of *Arachis hypogaea*.

There was one growth-promoting zone corresponding to the position of gibberellin A on the chromatogram of *Cytisus scoparius*. The histogram of *Sophora angustifolia* (Fig. 3-D) was similar to that of *Cytisus*. Although their growth activity near the position of gibberellin A was rather irregular, the extracts of *Phaseolus angularis* (Fig. 3-E) and *Vigna sesquipedalis* (Fig. 3-F) seem to belong to the type of the *Cytisus* histogram. This irregularity may be considered to be due to growth-inhibiting substances, which were also found in the extracts and lay close to the growth-promoting area on the chromatogram. As previously reported[2], the rice leaf growth-promoting substance in the bean (*Phaseolus vulgaris*) extract was found to have the same Rf value as that of gibberellin A. Very recently, MacMillan and Suter[4] announced the isolation, in pure form, of gibberellin A_1 from immature seeds of *Phaseolus multiflorus*. The four known gibberellins, A_1, A_2, A_3 and A_4, could not be distinguished from each other on the basis of Rf values in the solvent system used. Therefore, the substance responsible for the growth promotion in this zone must be either one of the gibberellins or a mixture of them.

On the other hand, only one growth-promoting zone lying in a zone of Rf 0.1–0.2 was detected in *Wistaria floribunda* (Fig. 3 I). The response of rice seedlings to the eluate from the zone near Rf 0.1 of its chromatogram is illustrated in Fig. 4. Rice seedlings treated with this eluate grew taller and their appearance was similar to that of gibberellin treated plants. As already mentioned, the four known gibberellins could not be separated from each other on the chromatogram and they were located near Rf 0.7 value. The Rf value of 0.1–0.2 of the active material from *Wistaria* differs from that of the known gibberellins. Therefore, the compound

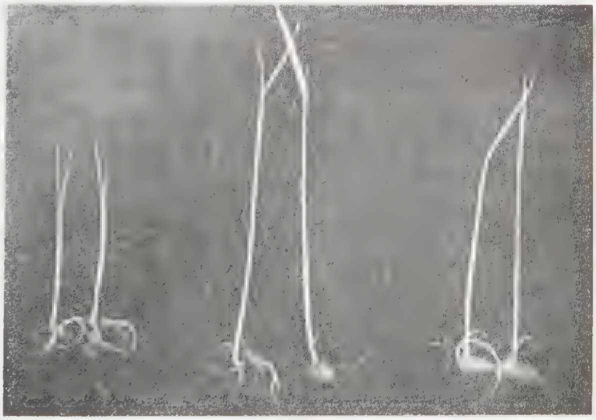

Fig. 4. Response of rice seedlings to gibberellin A and gibberellin-like substance from *Wistaria floribunda*.

Left, control; center, treated with the eluate from a chromatogram zone corresponding to Rf 0.06-0.12 in Fig. 3 I; right, treated with gibberellin A (0.5 mg./l.).

detected on the chromatogram and responsible for its growth-promoting activity on rice seedling may not be identical with any of these compounds. Immature seeds (of 8 mm. length) of *Maackia amurensis* (Fig. 3-J) contained considerably higher concentrations of a gibberellin-like substance similar to that of *Wistaria* and its content was almost equivalent at least to 5 μg. gibberellin A per gram fresh weight of the seed. Thus it is very desirable to elucidate its chemical nature. Similar histograms, which showed more or less lower growth-promoting activity, were also found with the extracts of *Albizzia Julibrissin* (Fig. 3-K), *Cercis chinensis* (Fig. 3-L). *Canavalia gladiata* (Fig. 3-M), *Pisum sativum* (Fig. 3-N) and *Medicago sativa* (Fig. 3-O).

The growth-promoting activity detected on chromatograms from extracts of seeds of *Glycine Max* and *Dolichos Lablab* was very weak as shown in Fig. 3-G and H. This does not necessarily imply the absence of gibbirellin-like substances, but the possibility cannot be excluded as to the presence of a much higher concentration of growth inhibitor on the paper chromatogram.

Fig. 5 shows the results of the auxin bioassay on the chromatograms of the extracts from the immature seeds of various plant species using *Avena* coleoptile sections. On every chromatogram of the extracts, except for that of *Albizzia Julibrissin* (Fig. 5-K), *Cercis chinensis* (Fig. 5-L) and *Medicago sativa* (Fig. 5-O), three growth-promoting zones were detected.

The central active area corresponded to the position of IAA marker spot. Despite of this, the presence in this area of substances with indole nucleus, as shown by the development of characteristic colour with Ehrlich's reagent, could be established only in the extract of *Canavalia gladiata*, *Glycine Max*, *Wistaria floribunda* and *Arachis hypogaea*, while the extracts from other species failed to give the positive reaction. The possibility remains that pigments contained in extracts might interfere with the development of colour.

A zone with considerable growth-promoting activity was also observed near the solvent front in every histogram. The Rf value was similar to those of indoleacetonitrile (IAN) or ethyl indoleacetate (EIA). On the paper chromatograms obtained from *Glycine Max*, *Pisum sativum*, *Arachis hypogaea*, *Robinia pseudo-Acacia*, *Medicago sativa* and *Sophora angustifolia*, a purple colouration with Ehrlich's reagent appeared near the region of Rf 0.9. No coloured spots could be detected on the chromatograms of other species. Fukui, Teubner, Wittwer and Sell[6] also observed that an auxin in the maize pollen, which had similar Rf value to that of IAN and EIA, gave colour reaction neither with Ehrlich's nor with Salkowski's reagents. It may therefore be considered probable that EIA and IAN are not the only growth substances responsible for the growth-promoting zone of Rf 0.9.

In addition, the third growth-promoting zone was noted near Rf 0.1. This promotion may be partly due to the presence of a substance for which Bennet-Clark and Kefford[7] have suggested the term accelerator α. Stowe and Thimann[8], after comparing chromatograms of synthetic indolepyruvic acid with those of a maize seed

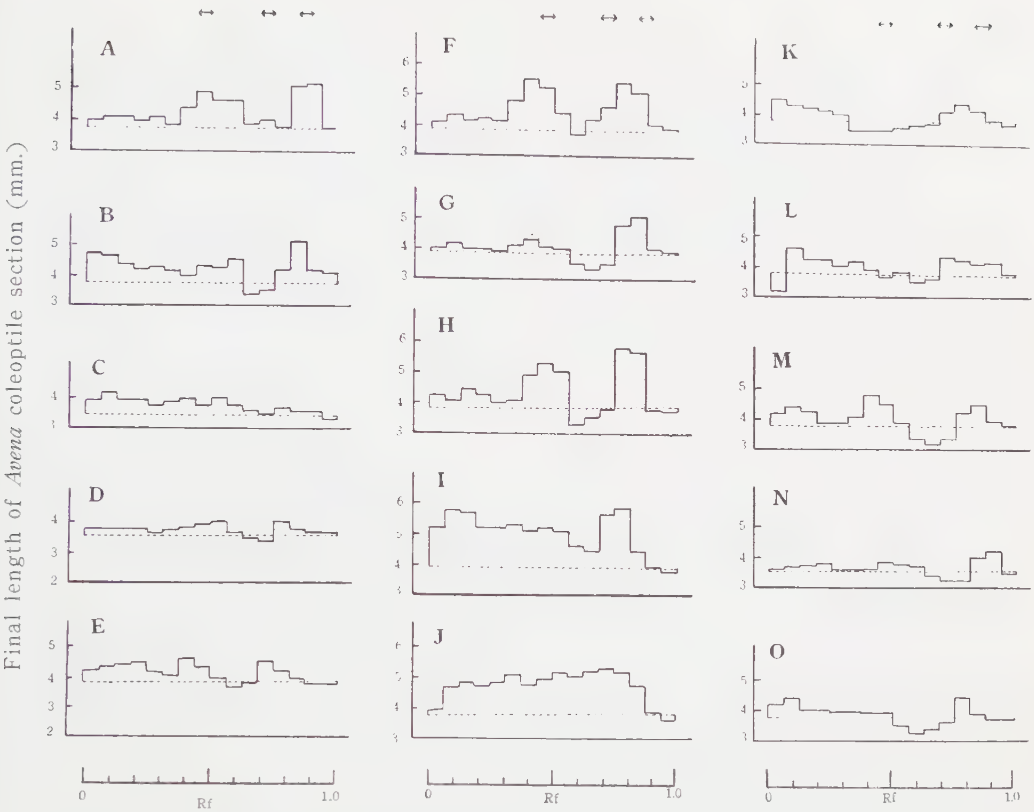

Fig. 5. Histograms showing auxin-like activity of ether extracts of immature seeds of Leguminosae after paper chromatographic development with ammoniacal isopropanol.

A, *Arachis hypogaea* (7 g.); B, *Robinia pseudo-Acacia* (4 g.): C, *Cytisus scoparius* (5 g.); D, *Sophora angustifolia* (2.5 g.); E, *Phaseolus angularis* (12 g.); F, *Vigna sesquipedalis* (10 g.); G, *Glycine Max* (3.5 g.); H, *Dolichos Lablab* (14 g.); I, *Wistaria floribunda* (1.6 g.); J, *Maackia amurensis* (4 g.); K, *Albizzia Julibrissin* (10 g.); L, *Cercis chinensis* (2.8 g.); M, *Canavalia gladiata* (3 g.); N, *Pisum sativum* (4 g.); O, *Medicago sativa* (1.6 g.).

Further explanations are as in Fig. 3.

extract, concluded that accelerator α and indolepyruvic acid are identical. However, other workers[9),10)] have not yet agreed with their conclusion. In this experiment it was difficult to detect a typical indole colour directly with Ehrlich's reagent on paper chromatograms, because a large quantity of coloured substances were present in this area.

A zone with Rf value greater than that of IAA showed inhibition of coleoptile section growth. It appears that the substance in this zone might be identical with the inhibitor β of Bennet-Clark and Kefford[7)]. As previously described, these regions also inhibited growth of rice seedlings.

The results of experiments above described indicate that not only auxins, but also gibberellins and growth inhibitors occur in the immature seeds of leguminous

plants. The occurrence of the activity similar to that of gibberellin was also found in the extracts of pea seedlings[11], inflorescences of *Brassica napus*[12], and suckers of *Citrus Unshiu*[13]. At the present time, it is always presumed that cell elongation in plants is controlled exclusively by auxins. Indeed, auxins induce growth of tissue sections excised from growing zones. They, however, have little effect on the elongation of intact plants. This in turn leads the writer to suspect that a growth factor or factors other than auxin may be operating in the plant growth regulating system. We now know that the gibberellins promote growth of intact plants and that they are produced naturally in higher plants. Thus the writer wants to assume that without gibberellin there is no elongation.

Summary

The distribution of gibberellins and auxins in immature seeds from 15 different genera of Leguminosae has been studied by paper chromatography.

Gibberellins were bioassayed by rice seedling method and auxins by *Avena* straight-growth test. Both gibberellins and auxins were detected in almost all seed extracts examined.

Two zones of gibberellin-like growth promotion were shown on the chromatograms developed in the mixture of isopropanol/water/ammonia (10:1:1). The activity of the one with Rf 0.7–0.8 was attributed to the known gibberellin A, while that of the other with smaller Rf value of 0.1–0.2, was due to a new gibberellin-like substance.

On the other hand, three zones of auxin-like growth promotion were present on the chromatograms. They had similar Rfs to IAA, IAN and the accelerator *a* of Bennet-Clark and Kefford, respectively. The zone with Rf of the inhibitor β of Bennet-Clark and Kefford inhibited the growth of rice seedling as well as that of *Avena* coleoptile section.

The writer wishes to thank Prof. T. Miwa for his kind revision of the manuscript.

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摘　　要

ジベレリンは高等植物にも含まれていることが最近明らかにされつつある。本報告では，マメ科に属する 15 種の植物の未熟な種子のエーテル抽出物について，ペーパークロマトグラフィーに生物検定法を併用し，ジベレリンおよびオーキシンの分布を調べた。アンモニア性イソプロピルアルコールで展開すると，二種のジベレリン類似の作用力がみられた。一つは，ジベレリンと同じ Rf 値であり，他は，既知のジベレリンとはちがつた Rf 値で，新しい物質である。オーキシンについては，IAA, IAN および "Accelerator α" に相当する Rf 値に作用がみられた。このように，ジベレリンは高等植物にも広く見いだされ，植物の伸長現象において，その役割の重要性が暗示される。

Formation of Starch in Isolated Chloroplasts III.

Behavior of Starch Grain in Separated Chloroplast after Restitution into the Cytoplasmic Mass Containing Nucleus

by Rikizo UEDA*

植田利喜造： 遊離葉緑体のデンプン形成 III. 有核原形質内へ復元された葉緑体におけるデンプン粒の変化

Received November 21, 1958

In my previous studies on the accumulation of starch grains in a detached leaf of *Elodea densa*, it was found that the chloroplasts, after isolation into the intra-cellular space intervening between cell wall and plasmolyzed protoplasm by means of plasmolysis, are still capable of producing starch grains, whereas the chloroplasts surrounded by the mother cytoplasm containing a nucleus scarcely produce starch grains even in the light (Ueda, 1949[1], 1958[2]). Similar observations were described by Yoshida (1956[3]) on the chloroplasts remained in fragmented cytoplasm within a single cell of *Elodea* leaf. According to him, in a cytoplasmic fragment containing nucleus, the chloroplasts become less and less active in starch formation resulting in a consumption of pre-existing starch grains, while in the cytoplasmic fragment lacking nucleus an active formation of starch takes place, until the chloroplasts are filled with starch grains in eight day culture.

From these observations, it is apparent that the *Elodea*-chloroplast continues to form starch grain even in the absence of nucleus, whereas it appears that the nucleus takes part chiefly in the digestion of accumulated starch grains (Ueda, 1958[2],[4]). In this connection, it is important to know whether or not starch grains formed in chloroplasts in the presence of non-nucleated cytoplasm can be digested, when the starch-containing chloroplasts are put back again into the mother cytoplasm with a nucleus. The present paper deals with some experiments carried out chiefly in order to answer this question.

Materials and Methods

Midrib cells in a leaf of *Elodea densa* were used throughout. In *Elodea* leaf cells treated with $CaCl_2$-solution, some of the midrib cells were easily plasmolyzed to form several chloroplast aggregates as well as cytoplasmic fragments within a cell (Fig. 1). In this experiment, $CaCl_2$–solution was used at the concentration of 0.2, 0.25, and

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0.3 M, respectively. In each plasmolyticum, *Elodea* leaves were cultured for several days under the fluorescent lamp (20 W) at 10 cm, distance (temperature: $30 \pm 2°$).

Fig. 1. Midrib cell of *Elodea densa* plasmolyzed with $CaCl_2$-solution, showing an aggregate of chloroplasts (left), a cytoplasmic fragment containing both chloroplasts and a nucleus (middle), and a cytoplasmic fragment containing chloroplasts but not a nucleus (right).

In a few days, chloroplasts alone or those with non-nucleated cytoplasmic fragment accumulated starch grains, whereas the chloroplasts in a nucleated cytoplasm became yellowish in color and did not form starch grains.

In order to recover the initial situation, the centrifugal force (12,500 rpm.) was applied to the plasmolyzed cell in its axial direction for 5 minutes (at 38°). Thereby, some cytoplasmic fragments fused together and the chloroplasts accumulating starch grains were driven into the nucleated mother cytoplasm. The same result was obtained on cultured cell as well by means of deplasmolysis using distilled water. In both cases, the treated leaves were cultured further under illumination with a fluorescent lamp (20 W) at 10 cm. distance (at $30 \pm 2°$). The behavior of each chloroplast was carefully examined under the microscope, and the starch was detected by iodine staining, and reducing sugar was measured with Fehling's reagent.

Results

The chloroplasts in a single cell, which were divided into several cytoplasmic fractions by means of plasmolysis, are almost identical with each other in microscopic appearance at an initial stage (Fig. 1). As time goes on, however, conspicuous dif-

Fig. 2. Midrib cell of *Elodea densa* plasmolyzed with 0.3 M $CaCl_2$-solution and cultured therein for 7 days. Aggregate of chloroplasts containing starch grains (left); starch-containing chloroplasts in non-nucleated cytoplasm (right); small, granulated chloroplasts in nucleated fraction of cytoplasm (middle).

ference comes into appearance among the chloroplast groups especially in their color, size, shape, and starch grains included. After several days, the chloroplasts, either

in an aggregated form or in non-nucleated cytoplasmic fragment, maintained their green color and produced an appreciable amount of starch, while the chloroplasts in nucleated mother cytoplasm became paler in color, smaller in size, and sometimes granular in structure, and did not form starch grains; moreover, pre-existing starch grains, if any, were completely digested (Fig. 2). In higher concentrations of the plasmolyticum used, the difference becomes more conspicuous. On treatment with 0.2

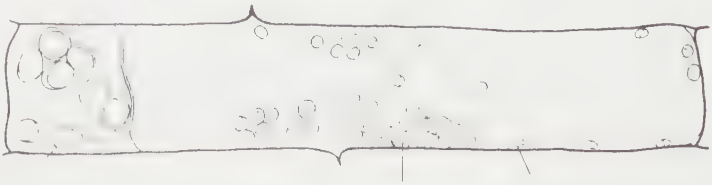


Fig. 3. Midrib cell of *Elodea densa* plasmolyzed with 0.25 M $CaCl_2$-solution and cultured therein for 7 days. Two cytoplasmic fractions initially separated come into contact with each other due to deplasmolysis, but do not fuse. The chloroplasts in the non-nucleated cytoplasm produce starch (left), whereas those remaining in nucleated mother cytoplasm do not form starch grains, but degrade altogether into granulated bodies (right).

to 0.25 M $CaCl_2$-solution, some of the separated cytoplasmic fragments were gradually deplasmolyzed and brought into contact with each other, but did not fuse (Figs. 3 and 4). Of course, further change could not be observed in both chloroplast groups in these cases.

Fig. 4. Photographic representation of a cell corresponding to the left half of Fig. 3.

After centrifugation, however, the remote or adjacent fragments of cytoplasm were sometimes fused altogether and the starch-containing chloroplasts were also put back into the mother cytoplasm. The same cytoplasmic recovery could be brought about also by means of rapid deplasmolysis using distilled water. By these techniques, a concomitance of large green chloroplasts and small yellow ones could be induced within a common cytoplasm, as shown in Figs. 5 and 6. Under the microscope, all plastids were found in movement in accord with the protoplasmic streaming.

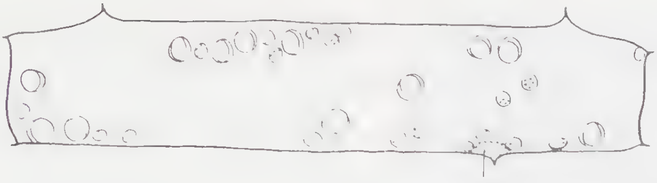


Fig. 5. Concomitance of two types of chloroplast in the cytoplasm admixed by centrifugation.

Consecutive observations on these chloroplasts obtained have revealed the following facts: Starch grains in the large, green chloroplasts are consumed thoroughly in two or more days even in the light, resulting in a conspicuous deformation of chloroplasts into dish-like bodies due to the loss of starch grain. These chloroplasts underwent a remarkable change in color and size, and degraded at last into yellowish green, granulated bodies. A diagrammatic representation of these results is shown in Fig. 7 (cf. Fig. 8).

Fig. 6. Photographic view of the chloroplasts shown in the middle part of Fig. 5.

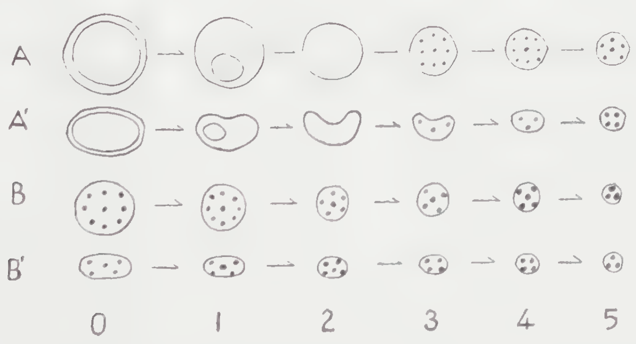
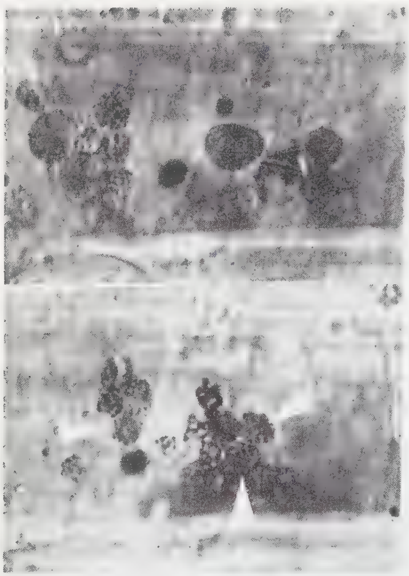


Fig. 7. Diagrammatic representation of the successive change in chloroplasts grown in a mixed cytoplasm. A: large and green chloroplast containing starch grain (top view), A′: lateral view of the above. B: small and yellowish green chloroplast having no starch grain (top view), B′: lateral view of B. The numerical figures at the bottom show the duration of culture in days after admixing.

In order to obtain further evidence for the conversion of starch into reducing sugar, the content of the latter was measured by Fehling's reagent on three kinds of cytoplasmic fragment; namely, nucleated, non-nucleated, and admixed cytoplasm.



The results were shown in Table 1, from which it is found that the amount of starch in chloroplasts is inversely proportional to that of reducing sugar present in the cytoplasm.

Fig. 8. Photographs showing the successive change of chloroplasts in the admixed cytoplasm. Top: large chloroplasts are green and contain starch grains, while the smaller ones yellowish green and granulated (taken after one day period after being admixed). Bottom: same cell as shown in the top figure. Green chloroplasts (arrowed) become smaller, somewhat granulated, and lose starch grains. Yellowish green chloroplasts become smaller, too. Photographed on the 5th day after the cytoplasm being admixed by centrifugation.

Table 1. Average amount of starch grains and reducing sugars measured in two types of cytoplasmic fragment and in an admixed cytoplasm. (−: none, +: minute amount, ++: small amount, +++: large amount, ++++: remarkably large amount of starch and reducing sugar, respectively).

Duration of culture (in days)	0	1	2	3	4	5	6	7	8
(a) Cytoplasm without nucleus									
Starch grain in chloroplasts	+	+	+	++	+++	+++	+++	+++	+++
Reducing sugar	+	+	+	+	+	±	±	±	−
(b) Cytoplasm including a nucleus									
Starch grain in chloroplasts	+	+	±	−	−	−	−	−	−
Reducing sugar	+	+	++	++	++	+++	+++	+++	+++
Continued from the above culture: Mixture of (a) and (b)									
Starch grain in chloroplasts*	+++	++	−	−	−	−	−	−	−
Reducing sugar	+++	+++	+++	++++	++++	++++	++++	++++	++++

* Chloroplasts derived from the fragment (b) do not recover the capacity of starch formation.

Discussion

In the above experiments with *Elodea*-leaf cells, it was shown that the chloroplast with or without cytoplasm has a capacity of accumulating starch grain in itself in the absence of nucleus, whereas in the nucleus-containing cytoplasm the starch grains appear to be consumed so rapidly that they are scarcely accumulated in

the chloroplast. Similar observation was also described by Yoshida (1956[3]). The starch grains in the chloroplasts are rapidly consumed, when these chloroplasts are replaced into the cytoplasm containing a nucleus. This indicates that the chloroplasts have an inherent property of starch formation, which is, however, veiled usually by nuclear activity directed preferentially to the digestion and successive utilization of starch. The nucleus plays, indeed, an important role in the digestion of starch grains primarily formed in the chloroplasts.

In parallel with the digestion of starch grains, the reducing sugar was found to increase gradually in the mother cytoplasm. The same fact was ascertained in the case of admixed cytoplasm, which was prepared by centrifugation or deplasmolysis from separated cytoplasmic fragments within a single cell.

In consequence, it appears that in *Elodea* leaves the chloroplasts are always going their ways to form starch grains on one hand, and the nuclei are contributing, in turn, to a successive degradation of assimilated starch into reducing sugar, so as to make it available for further metabolic transformations.

Summary

1. Using midrib cells of a detached *Elodea* leaf, the cytoplasm was separated, by means of plasmolysis, into two or more cytoplasmic fragments with or without nucleus, whereby several types of chloroplast aggregates were observed in a single cell.

2. In the absence of nucleus, either aggregated chloroplasts or chloroplasts in the cytoplasmic fragment remained green and accumulated starch grains, whereas the chloroplasts in the mother cytoplasm containing a nucleus showed the symptom of degeneration in size, color and structure, as well as the depletion of starch grains.

3. When the chloroplasts accumulating starch grains were brought into the nucleated mother cytoplasm by centrifugation or deplasmolysis, the starch grains in chloroplasts disappeared in a few days even in the light, while the chloroplasts outside the mother cytoplasm continue to produce starch grains.

4. With *Elodea* leaves it was demonstrated that the nucleus plays an important role in the digestion of starch grains in the chloroplasts.

I should like to express my sincere thanks to Prof. Tomoo Miwa in the Tokyo University of Education, and Prof. Bungo Wada in the University of Tokyo, for their invaluable suggestions and encouragements.

References

1) Ueda, R., Bot. Mag. Tokyo, 62: 62 (1949). 2) Ueda, R., ibid. 71: 366 (1958). 3) Yoshida, Y., Jour. Fac. Sci. Niigata Univ. Ser. 2, 2: 73 (1956). 4) Ueda, R., Bot. Mag. Tokyo, 71: 326 (1958).

摘　　要

1. 茎から切り離したオオカナダモの葉の中肋細胞を原形質分離させると，一つの細胞内で有核・無核の原形質片や葉緑体集団が得られる。

2. 無核の原形質片内の葉緑体や葉緑体集団は緑色のままで，核なしでデンプン粒を蓄積するが，有核の原形質片内の葉緑体は大きさや色や構造が変化し，しだいに退化してデンプン粒は消失する。

3. デンプン粒を蓄積した葉緑体を遠心力の作用や原形質分離復帰によつて，有核の母原形質内にもどすと，光があつても葉緑体内のデンプン粒は数日内に急速に消失する。

4. このような実験からオオカナダモの葉では，葉緑体内のデンプン粒の消化には核が重要な役割を演じていることが推考される。

Micrococcus glutamicus の細胞学的研究

第1報　形態学的形質ならびに核分裂について

板　垣　史　郎*・木　下　祝　郎*

Shiro ITAGAKI* and Shukuo KINOSHITA*: Cytological Studies on *Micrococcus glutamicus*. Part 1. Morphological Features and Nuclear Division.

1958 年 10 月 18 日受付

一般に，微生物の形態が，その life cycle において変化することはもちろんのこと，種々の生活環境の相異によっても変化することはよく知られた事実である。それゆえ，形態観察を行なうときには，微生物とその条件の関連に留意し，じゅうぶん慎重を期さなければならない。さらに一見ごくささいに思われる条件の変化に対しても，大きな形態変化を示す場合がよくあることは，とくに注意を要する。

木下等[1),2)]により分離，検討された *Micrococcus glutamicus* は，その培養経過にしたがって，かなり大きな形態変化を示し，一見，仮性根菌形態を示すことがある。この点に着目し，細胞学的検討を行ない，形態変化の要因を追究しようと，二，三の実験を行なった。その結果，本菌の形態変化は，不完全分裂に基因する多細胞形態の形成によるものであることを知った。

本報では，主として形態観察についてえられた知見をのべ，あわせて核分裂について報告する。

実験材料および実験方法

使用菌株： *Micrococcus glutamicus* 534

使用培地： 組成を次に示す

glucose bouillon 培地

glucose	20 g.	pH 7.0
Peptone	10 g.	
肉エキス	5 g.	
NaCl	2.5 g.	
水にて	1 l.	

合成培地

glucose	50 g.	$(NH_4)_2SO_4$	5 g.	pH 6.8～7.0
KH_2PO_4	0.5	尿素	5 g.	
K_2HPO_4	0.5	Biotin	2 γ	
$MgSO_4$	0.25	Phenol Red	15 mg.	
$FeSO_4$	0.01	水にて	1 l.	

培養方法： Shaking culture および Jar fermenter incubation によった。培養温度は 28° とした。前培養には glucose bouillon を用いて 24 時間行ない，合成培地に対して 10% の割合に加えた。

結　　果

1)　基礎的諸性質について

分類学的諸性状についてはすでに木下等[2)]の精細な報告があるので省略し，若干の色素に対する態度について述べよう。

a)　Ziehl-Neelsen の抗酸性染色では抗酸性が認められない。

b)　Lugol 液による glycogen および granulose の反応は認められない。

c)　Burdon[3)]の方法により fat droplet が認められる。この点に関して別に報告するつもりである。

d)　Leifson[4)]の方法により capsule は認められない。

e)　Janus green による mitochondria は認められない。この点はさらに検討するつもりである。

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f) Giemsa 染色では後述する methylene blue と同一所見がえられた。

2) 経時的形態変化の観察

培養各時期の菌体を遠沈で集め，pH 7.2 の phosphate buffer で洗浄し，常法にしたがって Löffler のアルカリ性 methylene blue 染色をほどこして検鏡した。電子顕微鏡観察も同時に行なったが，これについては第2報で述べる。

a) glucose bouillon 培養所見

24 時間培養菌体について観察した。菌体は類楕円形を呈し，中央部が濃染される。これは核と考えられるが，この点については後述する。全般的には glucose bouillon 中では菌形は整一であるが，異常長桿菌形態も認められる。このような長大菌には，通常の 4~8 倍ぐらいの長さを示すものもある。

glucose bouillon 培養所見を第1図に示す。

第1図　glucose bouillon 24時間培養 methylene blue 染色

b) 合成培地培養所見

glucose bouillon から合成培地に移植した直後では，前記 glucose bouillon 培養所見に酷似しているが，菌体の極附近に小顆粒の認められることがある。次に経時的に行なった観察の概要を述べる。（本小顆粒を以下極顆粒と仮称する。）

1.5~3$^{\mathrm{h}}$: 極顆粒はほとんど全細胞に認められ，それぞれ両極に存在する。本顆粒の色調は，菌体が青紫色を呈するのに，紫赤色がかって輝くように観察され，いわゆる metachromasy を示す。菌体構造中特異なものと考えられる。中央濃染部分は種々の形を示す。この時期の所見を第2図に示す。

第2図　合成培地3時間培養 methylene blue 染色

5~7$^{\mathrm{h}}$: 菌形は比較的整一であり，極顆粒はやや減少するが，大部分の菌体に認められる。菌形はやや伸長し，一見すると短桿菌の形態をもつようになる。中央濃染部分は後述する核染色所見に一致するので，この部分は核と推定される，核については後述するのでここでは省略する。

9~12$^{\mathrm{h}}$: 菌体はやや長大化し，細胞質全体の染色が強まるため，核部分の形や，極顆粒の存在は不明瞭になってくる。

15~24$^{\mathrm{h}}$: 菌体の染色性はだんだん強まり，菌体中央附近には菌体の長軸に対して直角方向にそまらない帯状部のある菌体が多くなってくる。これは後述する cell wall 染色所見とくらべると，septa の位置に一致すると考えられる。菌形は肥大桿菌形を呈し，極顆粒は認められない。

3) Cell wall 染色所見について

cell wall の染色は Webb[5] の方法にしたがった。分類学的検討の結果，本菌は *Micrococcus* に属すると考えられるようになったが，培養条件によって形態が変わり，長肥大桿菌形やあるいはさらに長い菌形を示す事実が本実験で認められるので，分類学上その意味は明確にされる必要がある。菌体が伸長する理由として，第一に培養条件に基因する分裂の不完全化が考えられる。本菌が glucose bouillon ではこのような長肥大桿菌形をほとんど示さないことからも，この点は考えうることである。しかし，本菌が合成培地で示すこの長肥大桿菌形態が正常な状態のものならば，分類学的位置も当然再検討を要することになろう。本実験は cell wall 染色により，長肥大桿菌形態の細胞に septa が存在するかどうかの検討を行なった。もし septa が存在しないとするなら，桿菌形態を示すことが本菌の重要な性質として，とくに検討されなければならない。また，septa が存在すれば，本来分裂，分離し，球菌形態を示すべきものが完全分裂を阻害する何らかの因子により，多数の細胞が連鎖したために一見して桿菌のように認めらたものと考えられる。このような観点から cell wall を染色して，その観察を行なった。

その結果について以下略述する。

a) glucose bouillon 培養

菌形は卵形ないし類楕円形が大部分であり，まれに septa のある菌を認めることができた。

b) 合成培地培養

ここに用いた試料は，methylene blue 所見と対比するために同一のものを用いた。

1.5$^{\mathrm{h}}$ ですでに 2~5 ケの septa を有する長桿

菌形態のものが認められた。とくに methylene blue で細胞質の染色性が強くなる時期にほぼ一致して，septa は明瞭となり，その数も増加する。20～24h 以降はほとんど全部の菌体に少なくとも 1 ヶ，最高 11 ヶの septa が認められた。大部分の菌体には，2～3 ヶの septa が存在する。この所見は 72h までほとんど変わらない。ごくまれに septa の認められない長桿菌形態の菌体が観察されることもあった。このような菌は，cell wall 染色によって菌体全体が比較的強くそまる。

以上の所見から，本菌がこのような培養条件で長桿菌形態を示すことは，不完全分裂の結果であるといえよう

代表的な cell wall 染色所見を第 3 図に示す。

biotin 含量が 0～5.0 γ/l. の間ではいずれもきわめて多数の septa が認められたが，0.5 γ/l. 以下では成育がわるい。

10～30 γ/l. では成育はきわめて良好で，septa を有する菌体はきわめて少ない。10 γ/l. では数ケの septa を有する長桿菌形態の細胞がまれに認められた。

以上のことから，本菌は 1 γ/l. ぐらいでもあるていど生育するが，不完全分裂を避け，ほぼ完全に mono cell の状態にするには，15 γ/l. ていど以上の biotin が必要である。

また，biotin を 10 γ/l. ていどまで増加すると，cell wall は Webb の方法ではきわめて染色されにくくなり，30 γ/l. になるとほとんど染色性を

第 3 図　cell wall 染色による septa の観察

c) Biotin 量と septa 形成の関係

本菌は biotin 要求株であるから，不完全分裂の原因は biotin の不足によるものかもしれない。前記合成培地に biotin 含量を変えて加え，そのさいの septa の形成を観察した。この結果を第 1 表に示した。

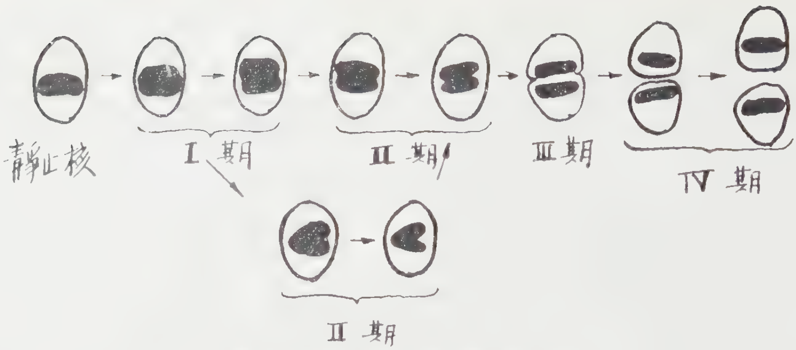
失なうようである。すなわち，biotin 量と細胞膜の化学組成との間には何らかの関係がありそうである。

4) 核分裂の観察

核染色方法は Robinow[6] の塩酸-Giemsa 法にしたがった。1N-HCl による加水分解は，検討

第 1 表　Biotin 量と Septa 形成の関係

Biotin 量 γ/l.	培養時間h	生　育	Septa 形成
10	24	きわめて良	きわめて少ない。まれに多数の septa を有する長桿菌形細胞を認めることがある。
	48	〃	
	72	〃	
15	24	きわめて良	きわめて少ない。
	48	〃	
	72	〃	
30	24	きわめて良	ごくまれ。
	48	〃	
	72	〃	



第4図　核分裂模式図

の結果 60° で 8～12 分行なうのが適当である。

種々の時期の菌体について，時期を追って核染色を行ない，観察した結果は，第4図に示すような核分裂模式図としてあらわすことができた。

本法で核観察を行なうには，1.5～3^h ぐらいのごく初期の菌体が適当である。methylene blue 染色所見で認められた中央濃染部分は核染色所見とほぼ一致する。また 15～18^h 以降，methylene blue で細胞質の染色性が強まる時期の菌体は，核染色材料としては不適当である。その理由は，第一に，この時期におそらく菌体中のリボ核たん白系の成分が増加するであろうことであり，第二は，この時期の菌体にはすでに多数の septa があることである。すなわち，みかけ上，1ケの細胞内に核染色を呈する部分が2ケ以上あらわれて，解釈が困難になる。

核分裂は通常細菌類に広く見られる無糸分裂の過程をへるものと考えられる。その概要を説明すれば，次のようになる。

I 期 (Prophase に相当): 静止核はまず膨大する。

II 期 (Metaphase に相当): 膨大した核は細胞の長軸に対して直角方向にくびれが入り，ついで2ケの相等しい核に分裂する。

III 期 (Anaphase に相当): 2ケの核ができあがると，この核の間にはきわめて薄い細胞質膜ができ，その間に cross wall が入りこむ。

IV 期 (Telophase に相当): 細胞は2分され，各娘細胞の一端にそれぞれ新生した核が存在し，この核はだんだん娘細胞の中心部へ移動し，分裂は完了する。

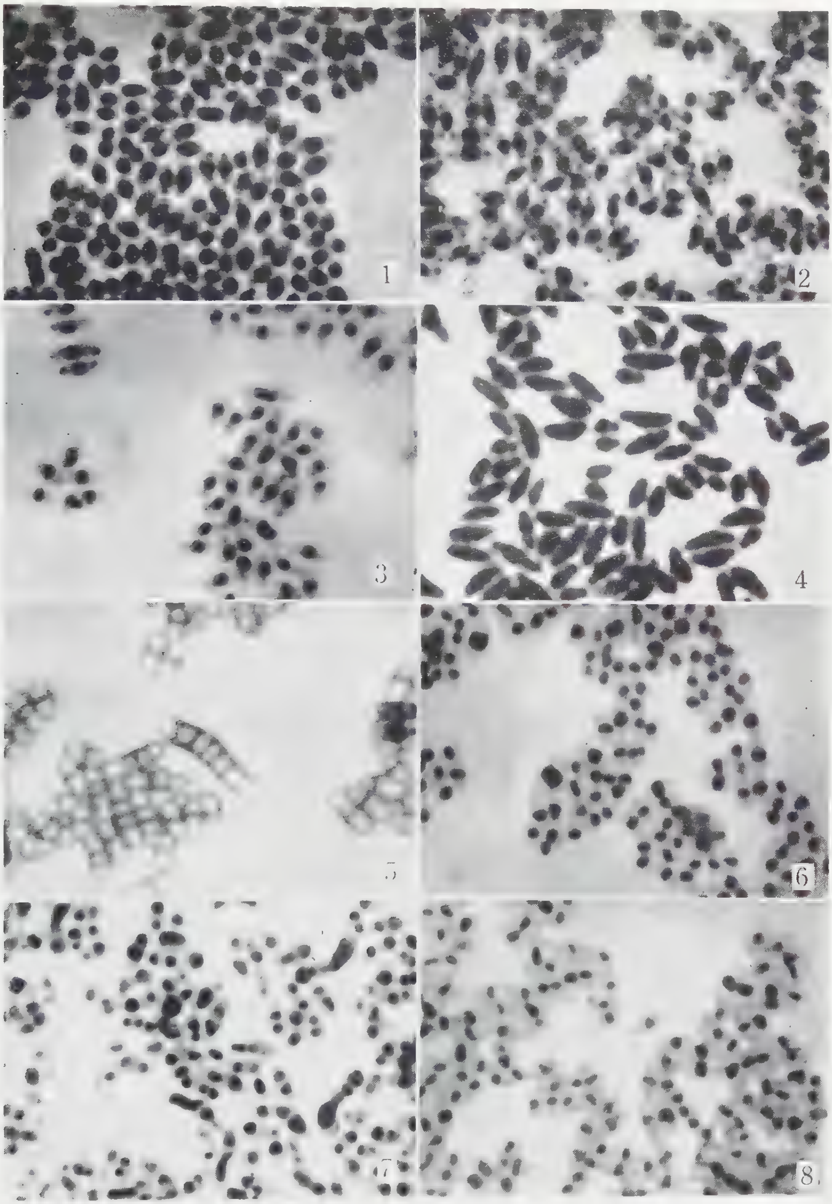
考　察

菌形態について

本 *Micrococcus glutamicus* は，合成培地で，培養の初期に菌形態をいちじるしくかえ，10数時間以降の培養では一見すると，長肥大桿菌形態を示す。この時期にはすでに数ケの septa が存在し，長肥大桿菌形態を示すのは，不完全分裂によって菌が連鎖したためであることを明かにした。この分裂阻害の原因については，第一に biotin の不足が考えられる。

本菌で連鎖と表現したような所見に関し，Bisset[7] は，fission より細胞分裂の方が早い場合にこのような multicellular になるとのべており，*Caryophanon latum* の写真を示し，"numerous, disk-shaped cells separated by cross walls" と説明している。しかし，*M. glutamicus* の場合はこのように細胞分裂が早すぎるためであるとは考ええない理由がある。この一つは，培養後期，すなわち stationary phase に入っても菌形態は変わらず，72^h にいたってもほとんど変化を示さないこと。第二に本菌が biotin 要求株であり，すでに述べたように，biotin を多量に添加すれば，菌体は個々に分離することである。

日下および北原氏[8] は，*Lactobacillus delbrückii* の分裂には Vitamin B_{12} が大きな影響をもち，菌の生長が最高となるためには，0.5 mγ/ml. であるが，この時は 10～100 μ の伸長形菌が高率に存在し，B_{12} を 1000 mγ/ml. に添加してはじめて伸長細胞はまったく生成せず，正常な 3～6 μ の菌体だけになる，と述べている。



1) Methylene blue 染色。glucose bouillon 24 時間培養。 2) Methylene blue 染色。合成培地 3 時間培養。両極に存在する極顆粒に注意。 3) Methylene blue 染色。合成培地 7 時間培養。 4) Methylene blue 染色。合成培地 24 時間培養。菌体は濃染され，長軸に直角方向に不染帯 (septa の位置に一致) がみられる。内部は不明になる。 5) Cell wall 染色。合成培地 24 時間培養。 6) 核染色。glucose bouillon 24 時間培養。 7) 核染色。合成培地 1.5 時間培養。6), 7) 共分裂各期の核を示す。 8) 核染色。合成培地 72 時間培養。みかけ上，1ケの菌体中に多数の核をもつ。[illegible]所見で[illegible]核分裂[illegible]考察[illegible]行[illegible] 不整菌形を示す菌体に注意。(写真倍率はいずれも 2000 倍)

このように，Vitamin や，諸種の抗生物質は微量であっても菌形態に与える影響は大きい。連鎖を形成することからみても，本菌の分裂様式は *Micrococcus* としてやや疑問をもたせる。Bisset[9] は Gram 陽性球菌の分裂についてのべ，*Micrococcus* の第二回目の分裂は第一回目のそれに対して直角方向に起こるとしている。このような分裂を行なう限り，連鎖を形成することはないはずである。さらに *Corynebacterium*[10] の分裂については，一方向に起きるため，multicellular を生ずるとのべている。このような細胞学的検討よりすれば，本菌は *Corynebacterium* に近縁なものではないかという疑問がもたれる。この点について，Bisset[11] は，微生物の evolution および classification の図を示し，*Actinomycetales* の枝では *Corynebacterium* は球菌に近いものもかなりあり，これがとなりの *Coccaceae* の cocci のところときわめて密接な関係があるといい，さらに cocci form の vaginal corynebacterium[12] の写真を示している。

中山等[13] は，本菌の菌体成分のなかに diaminopimelic acid が存在することをみいだした。*Micrococcus* には，従来 diaminopimelic acid は存在しないとされており，このことからみても本菌を *Micrococcus* に所属させることは，やや疑問に思われる。本菌は，菌形態を球菌と考えず，むしろ桿菌であると考えれば，*Micrococcus* とせずにむしろ *Corynebacterium* であるとすることができるかもしれないが，球菌と桿菌との間には種々の中間型が存在する以上，どこまでを球菌とし，どこからを桿菌とするかは，見解の相違にもとづくにすぎない。したがって現在の段階では，この問題はきわめて判定の困難なものの一つといえよう。

極顆粒について

本菌は，培養初期に methylene blue により特異的に染色される極在性顆粒をもっている。

このような極在性顆粒については，すでに多くの報告がある。DeLamater 等[14] や Mudd[15] は，このような顆粒は bacterial mitochondria であると主張しているが，Bradfield[16] はこれに反論をとなえており，現在はまだ一般細菌学者間の意見が一致していない時でもあるので，この顆粒に関してはあまり推論をのべることはさしひかえ，後日さらに検討を加えたいと考えている。

核分裂について

本菌の核分裂は，培養の初期にとくによく観察できる。このことから前述したような核分裂模式図をかいた。本菌はもっとも普通にみられる無糸分裂を行なうものと考えられる。しかしこのような分裂様式をとる場合，核そのものが単一染色体からなりたっている，という考え方もあるいは成立するのではないだろうか。

要　　約

木下等により発見され，*Micrococcus glutamicus* と命名された菌につき，若干の細胞学的検討を行なって二，三の知見をえた。

1. Methylene blue および Giemsa で染出される極在性の小顆粒が認められた。本顆粒は合成培地で培養することにより顕著に生ずるが，とくに培養の初期にはっきり認められる。
2. Cell wall 染色により長肥大桿菌形態のものは不完全分裂に基因する multicellular であることを確認した。
3. 合成培地において，biotin 量を $10\gamma/l$. 以上添加すれば完全分裂を行ない，ほとんど septa が認められない。
4. 本菌の核分裂は無糸分裂による。
5. 本菌は抗酸性をもたない。
6. Glycogen および granulose をもたない。
7. Fat droplet が認められる。
8. Capsule の存在は認められない。

謝　辞

種々御助言，御指導をいただいた東大教授湯浅明博士に深く感謝する。

Summary

Microorganism which was discovered and identified as *Micrococcus glutamicus* by Kinoshita *et al.* was studied from the view-point of cytology.

1. Small polar granules were observed when stained with methylene blue or

by Giemsa staining. These granules appear at the polar parts of cell when *M. glutamicus* was incubated in the synthetic medium. They were clearly observed at the early stage of growth.

2. Large rod-like cells were thought to be multicellular form due to incomplete cell division.

3. This organism does not produce septa in the synthetic medium when more than 10 γ/l. of biotin is added to the medium.

4. Nuclear division is thought to be amitosis.

5. This organism does not show acid-fast character.

6. Glycogen and granulose were not observed.

7. Fat droplets were observed clearly.

8. Capsule was not observed.

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抄　　録

仁内含有体の解釈

Serra, J. A., Interpretation of nucleolar inclusions. Nature, **181**: 1544—1545 (1958).

一般に細胞核内の仁の構造は一様であると思われていたが，1920 年頃から仁内に時には小胞や顆粒または擬晶体などが見られることが報告され，さらに 1950 年頃からは，仁には "*nucleolonema*" と呼ばれる繊維がもつれたような恒常的構造があることが明かにされ，近時は電子顕微鏡によってその微細構造がしだいに知られつつある。しかし一方その本体についてはなお疑問と考えるむきもあって，ある場合には仁染色体の仁部があたかも仁内繊維のように見えるのであろうといわれ，またある場合には仁内に小胞や顆粒などが形成され，それらの癒合によってヌクレオロネマ様の構造をあらわすのであろうともいわれ，実際ある条件のもとでは仁が顆粒や糸状物に分解することなども知られている。種々の植物の根端分裂組織では，すべての仁にうねりくねったヌクレオロネマが明かに認められたが，一方蛇類の卵母細胞やユスリカの唾腺細胞などでは一般に典型的な，いわゆるヌクレオロネマは示さないか，あるいはごくまれであった。いろいろの視点からみてこのヌクレオロネマというものは，結局，仁内の小胞あるいはその他の仁内分化産物と同列のものであって，仁の中で phase（相）の分化として生ずる phase の部分的分離が interphase（内相）をつくり，それが vacuoles（小胞）に発達し，これが固定・染色等の人為操作の影響によっていわゆるヌクレオロネマ様構造として可視的になるものであると思われ，その関係はちょうど細胞質内のゴルジ体における状況と同様である…むかしは特定の機能をもった恒常器管と思われたが，近時は Phospholipid-phase（燐脂質相）の現わす人為像といわれる。要するにヌクレオロネマもまた，仁内永久構造でも必須のものでもなく，ある phase の現わす artifact である。　　（吉田吉男）

ミズカビ Allomyces の誘引性ホルモン "サイレニン" の研究

Machlis, Leonard, A study of sirenin, the chemotactic sexual hormon from the watermold *Allomyces*. Physiologia Plantarum, **11**: 845-854 (1958).

ミズカビに属する *Allomyces macrogynus* と *A. arbuscula* との雑種の雌性の配偶子から，雄性の配偶子を誘引するホルモン "サイレニン sirenin" が分泌されることについては，著者が Nature (181, 1958) と Physiologia Plantarum (11, 181, 1958) に報告をだしている。これによれば，このホルモンは透折膜を通過し，脂肪溶媒にとけ，光に安定，中性溶液ならば 100° に 1 時間熱しても分解しないとされている。著者はこのサイレニンを精製して，紫外線および赤外線スペクトル分析，質量スペクトル分析，元素分析の結果をこの論文で報告している。

雌性配偶子の液をザイツ濾過器で濾過し，サイレニンを炭末に吸着させ，クロロフォルムで溶離させ，クロロフォルムを蒸発させたのち，水で抽出し，水を蒸発させたのち，もう一度クロロフォルムに抽出する。これを珪酸の柱に通して分割する。こうして作った標品は 1 l. につき 10 μg. という濃度で雄性配偶子に対して吸引効果がある。

サイレニンは分子量として約 400 をあたえ，また $C_{21}H_{36}O_7N$ に相当する元素分析値をあたえる。中性であり，芳香族に属しない。ラクトン環をもち，ケトン，アルデハイド，メトキシル基の反応を呈する。

以上のような結果から判断すると，サイレニンはまだ完全に純粋な標品になったというわけではないし，芳香族に属しないにもかかわらず，いろいろな官能基の存在を示すことから，なかなか構造のむずかしい物質であるように思われる。

（服部静夫）

根端におけるプロトクロロフィル

Hejnowicz, Z., Protochlorophyll in root tips. Physiol. Plantarum, 11: 878–888 (1958).

著者はスウェーデンのルンド大学植物生理学教室（教授 H. Burström）所属の人であるが，今までに報告されたことのない，赤いプロトクロロフィル *a* の存在することを発見した。この研究は暗所でそだてられた根の先端に強い青色光線をあてると，はっきりした赤い螢光のでることをみいだしたことにはじまっている。従来，根の先端に白，黄または青い螢光を発する物質のあることはLinsbauer (1929), Goodwin and Kavanagh (1948), Goodwin and Pollock (1954), Eberhardt (1955) などによって報告されているが，赤い螢光を出す物質のあることは報告されていない。

コムギの根にナフタリン酢酸をあたえて生長を阻害させると，光のなかにおいたばあいには，皮層の細胞から強い赤い螢光をだすが，これがプロトクロロフィルムにもとづくことは Burström and Hejnowicz (Kungl. Fysiogr. Sällskapets i Lund Förhandlingar, 28, 65 (1958)) によってあきらかにされている。しかし，暗所でそだてると，根の先端の細胞分裂のさかんな部分に赤い螢光がでるようになる。これは約 430 mμ の光でもっとも強くおこり，その螢光の波長は約 630 mμ にある。

シダ 4 種，裸子植物 5 種，被子植物 8 種についてもしらべたが，どれでも同じ結果がえられたから，この現象はかなり普遍的なものだろうという。

もちろん，プロトクロロフィル *a* の植物器官内での含量はきわめてわずかであるから，Aminco-Bowman 螢光スペクトロフォトミータで曲線をとっただけである。

プロトクロロフィルがなぜ，また，どのようにして形成されるか，またその生理学的意義はなんであるかについて著者は若干の議論をしているが，これは無論将来の問題である。　（服部静夫）

訂　　　正

Berichtigungen zur Arbeit von C. Kujirai und S. Imamura im Bd. 71, 408–416, 1958.

Seite 409, Zeile 23: statt Abhnägigkeit lies Abhängigkeit.

Seite 410, in der Tabelle 1: statt Lage der ersten Blüten lies Lage der ersten Blüte.

Seite 411, in der Erklärung der Tabelle 2: statt Violett lies Violett und Tendan.

Seite 412, in der Tabelle 4: statt Bluhquotient lies Blühquotient.

Seite 413, in der Tabelle 5: statt Bluhprozent lies Blühprozent.

Seite 414, in der Erklärung der Tabelle 6: statt Asugesät lies Ausgesät und in der Erklärung der Fig. 1: statt Kotyledonarbüten lies Kotyledonarblüten.

一月号掲載の本会記事の評議員選挙の記事の中に東北支部の定員 1 名は 2 名の誤りですから訂正いたします。

本　会　記　事

支部通信

関東支部

1月例会（1月24日，於東大・植）小林義雄，椿啓介：オニゲナ菌とその培養。服部静夫，野口市夫，吉田精一：霊菌のクロモフロテイドについて。

中部支部

第55回例会（1月31日，於愛知県立女子大生物）岩崎秀一：思考について（論文紹介）。熊沢正夫：ショウジョウバカマの葉上不定芽の組織発生。

九州支部

第52回例会（11月29日，於九大，理）渡辺皓：スイバのB染色体。小野林：米国植物学界の現状。

第6回熊本例会（11月15日，於熊本大）藤木素士：樹木苗に含まれる金属イオンとその分布。乙益正隆：熊本県内で最近発見されたシダ植物。野口彰：植物の隔離分布。

第53回例会（2月7日，於九大・理）山永立木：福岡城跡のツクシオオカヤツリなどの群落分布とその保護について。江頭威：ハンアカカビにおける二，三の遺伝学的観察。

なお九州支部の新役員はつぎのようになりました。

(1) 支部長　千葉保胤

(2) 委員　細川隆英，瀬川宗吉，芳賀忞，野口彰，千葉保胤

(3) 地区委員
佐賀：村山宅美，長崎：外山三郎，大分：鈴木時夫，宮崎：服部新佐，鹿児島：山根銀五郎

新着雑誌のおしらせ

1957年9月以降，現在までに新たに交換をはじめた外国雑誌は下記の通りです。

Acta Biologica Venezuelica
Acta Cientifica Potosina
Annuaire de la Faculté d'Agriculture et de Sylviculture de l'Université de Skopje Agriculture
Australian Journal of Botany
Canadian Journal of Botany
Journal of Chemical Education
Notulae Systematicae ac Geographicae Institute Botanic Tphilisiensis
生理学報
植物学文献
Senckenbergiana Biologica
Wissenschaftliche Zeitschrift der Karl-Marx-Universität Leipzig

学会図書閲覧規定

1） 閲覧者は日本植物学会会員にかぎる。

2） 閲覧者は学会事務室備つけの帳簿に氏名，住所または所属機関（できるだけ詳細に），図書名，借出の日づけを記入したのち図書を受けとること。

3） 借出期間は2週間とする。

4） 四返還する時は図書を事務室に持参し，帳簿に返還の日づけを記入すること

5） 汚損，紛失した時は，補修，新規購入の費用を負担すること。

付　記

図書カードは東京大学理学部植物学教室図書室にあります。

外国雑誌に関しては70巻831号（1957年）に目録がありますので御参照ください。

Electron-microscopical Study on Fine Structures of Diatom Frustules. XVII
Stereoscopic Observation

by Haruo OKUNO*

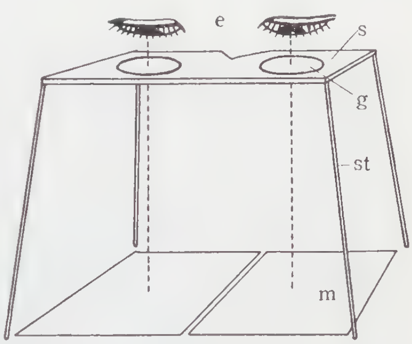
奥野春雄*: 電子顕微鏡による珪藻殼微細構造の研究. XVII
立体写真法による観察

Received November 25, 1958

Introduction

In research of the three-dimensional character of the fine structure of diatom frustules, it is very helpful to prepare the stereoscopic electron micrographs[1] of them.

By this reason, I will describe the fine structure of the diatom frustules presenting their electron stereomicrographs. You can easily set up a stereoscope by fixing a pair of magnifying-glasses of unity power of about two magnifications at the same distance apart as your two eyes. View each pair of the stereomicrographs presented here through a stereoscope held close to your eyes, then you can get a sharp stereo-image of the micrographs (Text-fig. 1).



Text-fig. 1. Schema for stereoscopy. e, Eye. g, Magnifying-glass. m, Stereomicrograph. s, Stereoscope with parallel visual axes. st, Stand.

When the pair of stereomicrographs presented here is viewed through such a stereoscope with parallel visual axes as shown in Text-fig. 1, the stereo-image will be exactly the same as the explanation "inside view" or the "outside view" in the Plates. On the other hand, if the stereo-pair is viewed through a stereoscope with crossed visual axes, the stereo-image will be opposite to the explanation. Further, you can get a stereo-image from the micrographs by the following way without the help of a stereoscope. Place a pair of stereomicrographs about 30 cm. from the eyes and try to see your own nose first. In doing that you will be able to make the visual axes of your eyes cross. Keeping the position of eyes in that way, try to see the micrographs and bring them near or take them away from eyes until you can get a sharp stereo-image of them. In

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doing such adjustment always try to keep your eyes cock-eyed. In this way by the naked eyes, the stereo-view will be opposite to the explanation as in the previous way.

Description of fine structure

Coscinodiscus Wailesii Gran and Angst (Text-fig. 2A; Pl. I, Figs. 1, 2)

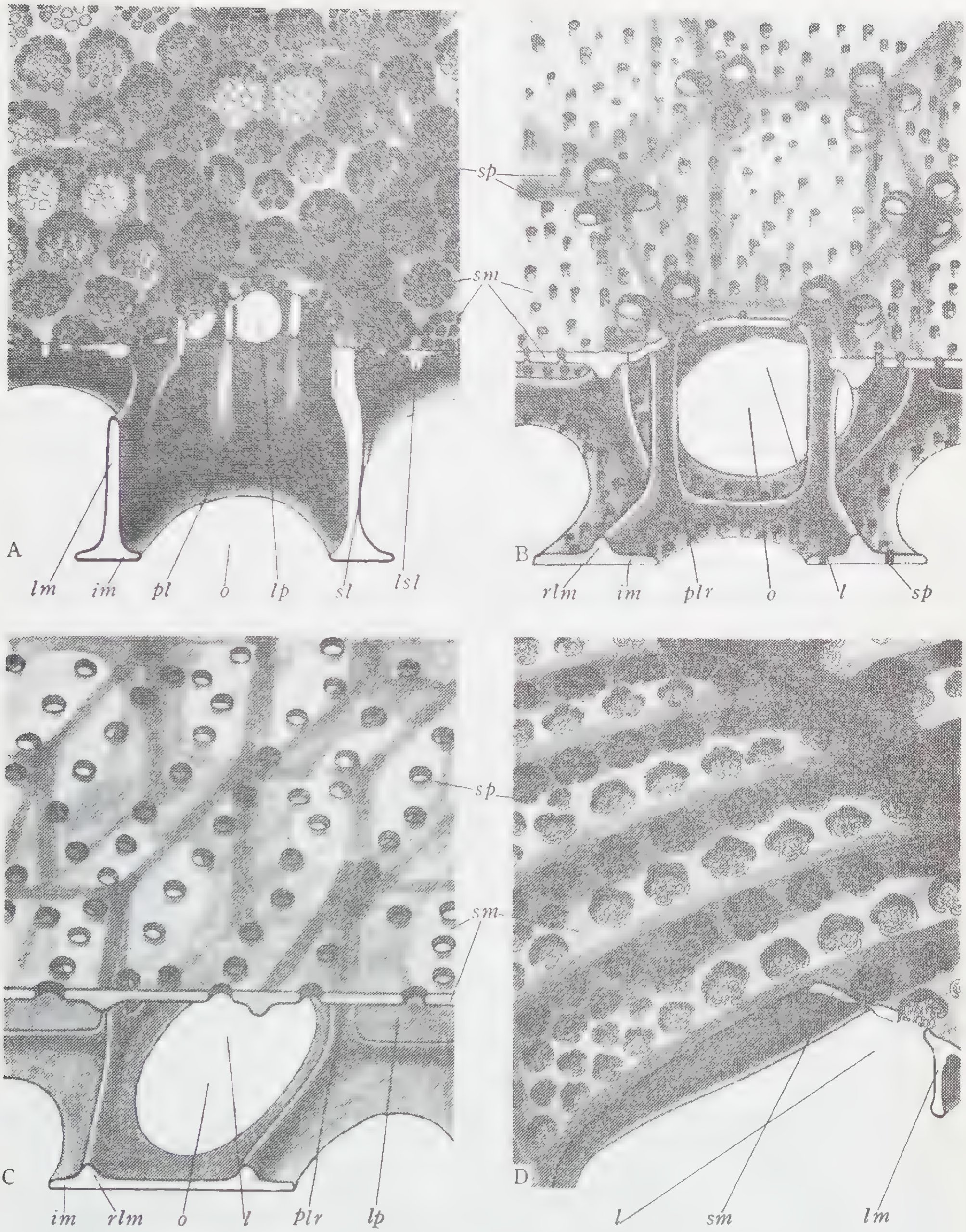
Syn. *Coscinodiscus Janischii* A. Schmidt, Okuno, Bot. Mag. Tokyo, **62**: 137, pl. 4, Figs. 4-6 (1949), **65**: 160, pl. 1. Figs. 1a-1b′ (1952); Okuno and Kurosawa, Bull. Fac. Text. Fib. Kyoto Univ. Ind. Arts and Text. Fib. **2**: 49 (1957).

I have already reported some electron-microscopical fine structure of the frustule of the present species (Okuno, 1. c.). Sorry to say, the plane micrographs published in my previous papers were insufficient to show the three dimensional structure of the frustule walls. In the present electron stereomicroscopy, the three dimensional structure of the loculus was clearly discerned. Loculi both on the valve surface and on the mantle are usually hexagonal, about 1.7–1.8 μ long and 1.5–1.7 μ broad, each closed outwards by a sieve membrane and half closed inwards by an inner membrane. The upper part of the lateral membrane on each side of the loculus is perforated by about 2 roundish lateral pores, through which the neighbouring loculi can communicate each other. The lower part of the lateral membrane is non-porous. The outer sieve membrane is very thin, perforated by fine rounded sieve pores (about 100 mμ in diameter) and each of which is divided into 2 to 4 micropores or meshes. Sieve membrane is backed inwards by a network, which at the same time represents the lateral membranes of the secondary loculi. The meshes of the network round or rounded polygonal, about 100–400 mμ in diameter and 6–15 in a loculus. The inner closing membrane of the primary loculus narrow, about 100–150 mμ broad, and the opening of the inner membrane about 1.0–1.7 μ in diameter. The dots near the edge of the mantle which described by Cupp as "spinulae"[2] were elucidated in the present electron microscopy to be the small loculi penetrating the frustule wall (Pl. I, Fig. 1). Those small loculi or interlocular pores, about 400 mμ in diameter, open almost freely in- and outwards without distinct closing membranes. The longitudinal lines starting from the small loculi, which described by Cupp as "hyaline lines"[2] were elucidated to be the common extension of the inner membranes of the loculi in the two neighbouring rows.

Habitat: Marine plankton. Seidan-chô, Awaji, Hyôgo Prefecture (Okuno, No. m1260. Oct. 1956).

Rhizosolenia styliformis Brightwell var. **latissima** Brightwell (Text-fig. 2B; Pl. I, Figs. 3, 4), Okuno, Journ. Jap. Bot. **27**: 352, pl. 2, Figs. 2–2‴ (1952); Okuno and Kurosawa, Bull. Fac. Text. Fib. Kyoto Univ. Ind. Arts and Text. Fib. **2**: 56 (1957).

I have already reported some fine structure of the frustule wall of the present variety observed by electron plane-microscopy[3]. By the present electron stereomicroscopy, the more detailed fine structure of the frustule wall was discerned. The



Text-fig. 2. Diagrams of the fine structure of loculi, reconstructed from the stereoscopic electron micrographs. A, *Coscinodiscus Wailesii*. B, *Rhizosolenia styliformis* var. *latissima*. C, *Biddulphia sinensis*. D, *Achnanthes longipes*. (*im*, Inner membrane. *l*, Loculus. *lm*, Lateral membrane. *lp*, Lateral pore. *lsl*, Lateral membrane of secondary loculus. *o*, Opening of inner membrane. *pl*. Primary loculus. *plr*, Pillar. *rlm*, Rudimentary lateral membrane. *sl*, Secondary loculus, *sm*, Sieve membrane. *sp*, Sieve pore.)

loculi on the intercalary bands hexagonal, rarely quadrate or pentagonal, about 500 mμ in diameter and about 20 in 10 μ, each with an outer sieve membrane and an inner half closed membrane. The outer and inner membranes are connected only at the corners of the loculus by rounded triangular pillars, leaving a large lateral pore at each side of the loculus. Judging from the present stereomicrographs, the height and the diameter of a loculus are nearly equal. The sieve membrane of the present specimen has six round or angular sieve pores (about 50 mμ in diameter) at the corners, and further has scattered minute pores of various shapes and sizes over the whole surface. The inner half closed membrane, about 100–150 mμ broad, with a large round opening about 200–300 mμ in diameter. In the presented specimen, the inner membrane was not only perforated by scattered sieve pores, but also distinctly spongy porous. Working the electron microscope at 50 KV, I could find such a spongy porous structure of the frustule wall in the whole surface of the frustule of *Chaetoceros affinis* (Text-Fig. 3), and in thin parts of the frustules (inner and outer membranes of loculi, edges of mantles and intercalary bands, and walls of setae) of some species of *Biddulphia, Cocconeis, Coscinodiscus, Eucampia, Melosira*. According to Helmcke and Krieger's electron micrographs, the frustules of *Podosira stelliger, Actinocyclus Ehrenbergii, Meridion circulare, Nitzschia dissipata*[4] are also spongy porous. If we use the electron microscope of much higher voltage, we shall be able to find such spongy structure even in the thick frustules which appear non-porous membraneous only by the insufficient penetration of the electron beam at such a voltage mentioned above. Further, I suppose the spongy structure of the frustule walls is a common character of Diatomaceae, which contributes to decrease the specific gravity of the frustule, and to increase the pore and space for metabolism. The loculi on the calyptrae usually quadrate, with more or less irregularly porous sieve membranes. The inner opening of the loculus elliptic to quadrate. The light-microscopically so called "Abdrücke der Schwesternschalen"[5] on the calyptra is elucidated to be a grove-like depression of the frustule, in which the seta of the adjacent frustule is inserted. The wall of the depression is porous with loculi as in the other parts of the frustule. Helmcke and Krieger published a pair of electron stereomicrographs of *Rhiz. styliformis*[6], in which the height, the pillars, and the lateral pores of loculi are clearly shown, but in them, we can not find any fine structure of the inner and outer membranes.

Habitat: Marine plankton. 50 miles south-east off the island of Tsushima (Okuno, No. m 1000. Jun. 1955. Collected by H. Maeda).

Chaetoceros affinis Lauder (Text-Fig. 3), Okuno, Bot. Mag. Tokyo, **69**: 186, pl. 1, Figs. 1–5 (1956); Okuno and Kurosawa, Bull. Fac. Text. Fib. Kyoto Univ. Ind. Arts and Text. Fib. **2**: 48 (1957).

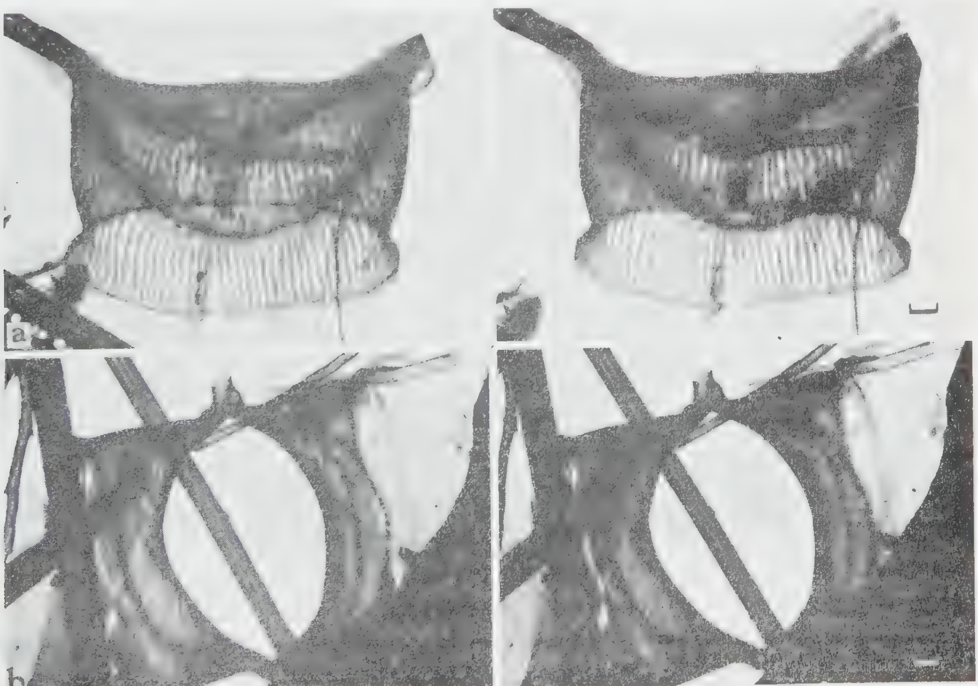
Valves elliptical. Valve surface saddle shaped, with slightly prominent radial ribs about 3–5 in 1 μ. Breadth of a rib about 100–200 mμ. The terminal valve with a central spine about 800 mμ long and 200 mμ in diameter, and scattered with minute

spinules about 500 mμ long and about 100 mμ in diameter. The central spine and the spinules are hollow, with spongy porous wall. The inner valve with a somewhat concave elliptical central area about 4 μ in diameter. The mantle line distinctly thickened. Mantles with longitudinal rows of ribs about 3-5 in 1μ. Intercalary bands with longitudinal rows of delicate ribs about 7-8 in 1 μ. The valve is scattered with rounded holes about 50-150 mμ in diameter, and further spongy porous as in the intercalary band. Setae hollow, about 1 μ in diameter, armed with spinules about 300–400 mμ long. Wall of the seta is porous with rounded holes. Holes about 50 mμ in diameter, 12–15 in 1 μ, arranged in spiral rows about 5 in 1μ.

Habitat: Marine plankton. Shimonoseki, Yamaguchi Prefecture (Okuno, No. m859. May 1953. Collected by H. Maeda).

Eucampia zoodiacus Ehrenberg (Pl. II, Fig. 1), Okuno, Bot. Mag. Tokyo, **63**: 99, pl. 2, figs. 3, 3′ (1950); Okuno and Kurosawa, Bull. Fac. Text. Fib. Kyoto Univ. Ind. Arts and Text. Fib. **2**: 51 (1957).

Central area of the valve distinctly concave, about 2–3.5 μ in diameter, with 2 to 4 central loculi. Valve surface with radial rows of loculi about 1–2 in 1 μ, and scattered with minute interlocular pores. A loculus rounded rectangular or polygonal, about 300–600 mμ in diameter, with a lateral membrane slightly prominent both in- and outwards, and closed by a porous sieve membrane. Sieve pores polygonal, about 5–7 in 1μ, and 5–25 in a loculus. I published a diagram of the loculi of this species, in which the inner closing membrane was shown by presumption[7], but in the present



Text-fig. 3. Pairs of electron stereomicrographs of *Chaetoceros affinis*. a, Terminal valve. b, Inner valves. Scales: 1 μ.

stereoscopy, it was discerned that the loculi have not such inner closing membranes. Interlocular pores round, about 30-100 mμ in diameter, opening freely in- and outwards without closing membranes. Intercalary bands very thin, with longitudinal rows of round or rectangular holes about 100-200 mμ in diameter, and about 3-3.5 in 1μ. The edge of the intercalary band adjoining the mantle, attaches to the inside of the mantle. Thin parts of the frustule, for example, the edge of mantles, intercalary bands, and the ridges (about 300-400 mμ high) on the blunt top of the apical processes, show the spongy porous structure.

Habitat: Marine plankton. Tomogashima, Wakayama Prefecture (Okuno, No. m613. Mar. 1952).

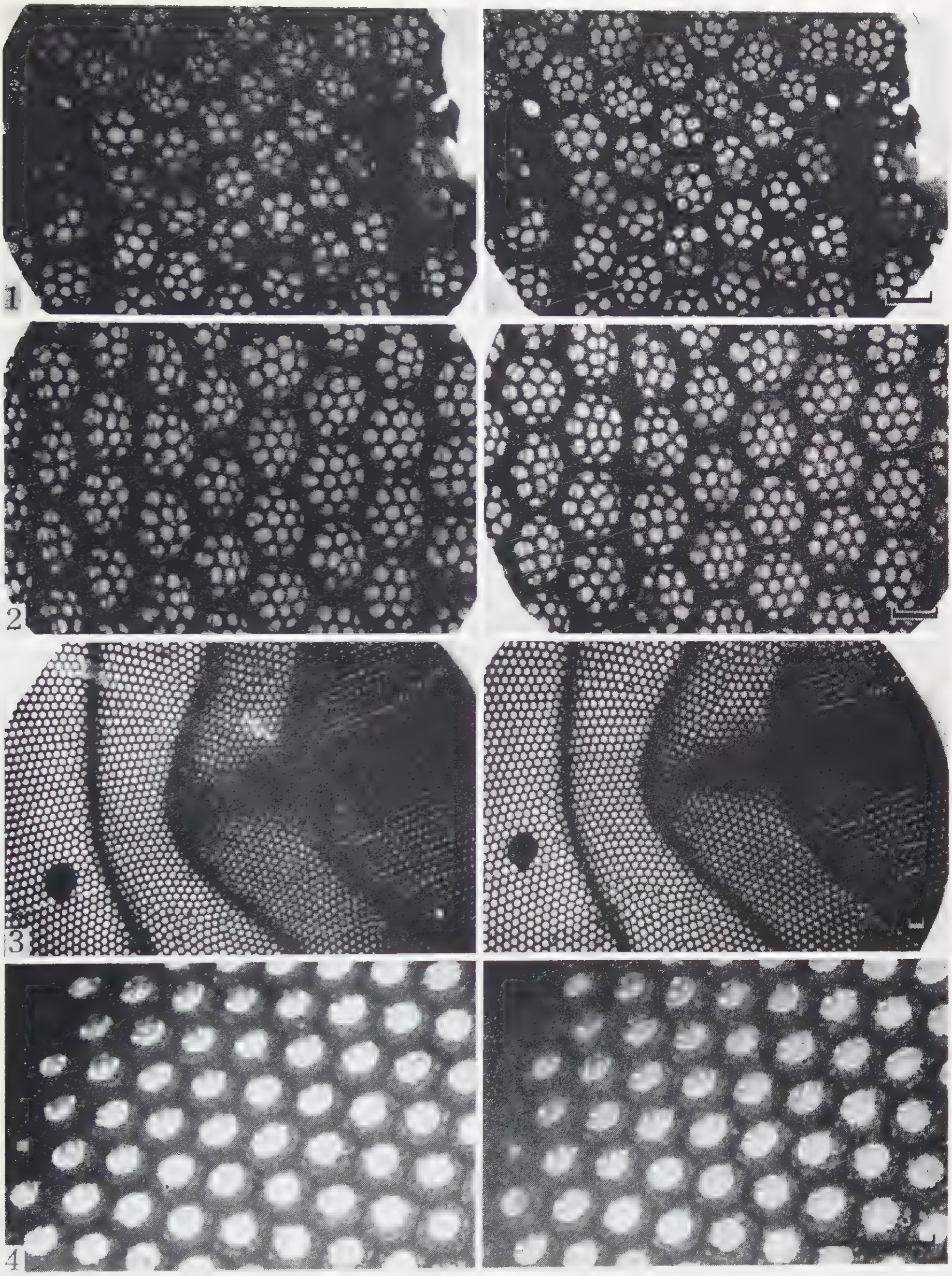
Biddulphia sinensis Greville (Text-Fig. 2C; Pl. II, Fig. 2), Kolbe, Ark. f. Bot. **33**: A: no. 17, p. 10, pl. 3, figs. 5-6 (1948); Okuno, Bot. Mag. Tokyo, **65**: 161, pl. 1, figs. 4a-4" (1952); Okuno and Kurosawa, Bull. Fac. Text. Fib. Kyoto Univ. Ind. Arts and Text. Fib. **2**: 48 (1957); Desikachary and Bahadur, Trans. Amer. Micr. Soc. **73**: 276, Fig. 4 (1954).

Loculi about 12-15 in 10 μ, arranged in radial (on the valve) and longitudinal (on the intercalary band) rows, about 15-20 in 10 μ. A loculus rhombic hexagonal, about 500-800 mμ long and 400-750 mμ broad, each closed outwards by a sieve membrane and half closed inwards by an inner membrane. The sieve membrane very thin, with longitudinally arranged peripheric sieve pores about 60-70 mμ in diameter and 6-9 in 1μ. The inner membrane, about 150 mμ broad, with a round to elliptic opening about 300-600 mμ in diameter. In the present stereoscopy, it was elucidated that the inner and outer membranes of the loculus are connected only at the corners of the loculus by the delicate pillars, leaving a large lateral pore on each side of the loculus. The pillars, at their base, somewhat triangular. The rudimentary lateral membrane of the loculus is found only at the inside of the outer sieve membrane. Intercalary bands imbricate by their edges with dentiform spines (cf. Okuno, l. c. p. 106, pl. 2, fig. 1c). The loculi, at their inside, are sometimes backed with a common non-porous membrane.

Habitat: Marine plankton. Seidanchô, Hyôgo Prefecture (Okuno, No. m991. Aug. 1954).

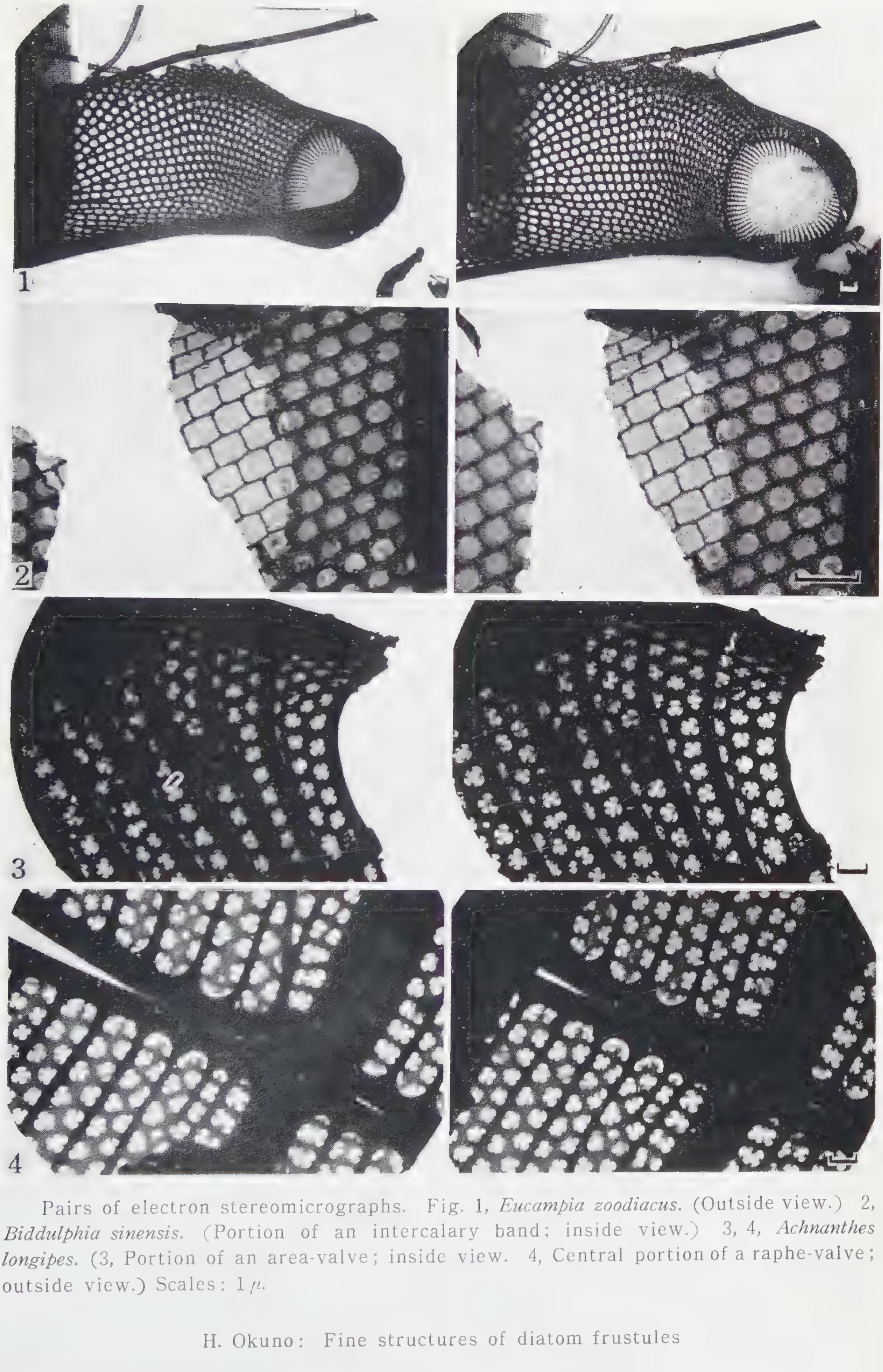
Achnanthes longipes Agardh (Text-Fig. 2D; Pl. II, Figs. 3, 4), Okuno, Bot. Mag. Tokyo, **66**: 6, pl. 2, Figs. 1a-b″ (1953); Okuno and Kurosawa, Bull. Fac. Text. Fib. Kyoto Univ. Ind. Arts and Text. Fib. **2**: 46 (1957).

In general structure, the loculi both on the raphe-valve and on the area-valve are the same. Loculi are transversely elongated, each closed outwards by a porous sieve membrane and opens freely inwards. In the present electron stereomicroscopy, the existence of the thin, non-porous lateral membrane of the loculus was clearly discerned. A loculus is highest in its middle part, and decreases its height both to the margin and the apical plane of the valve. The sieve membrane of a loculus with 2-4 transverse rows of sieve pores, in each row, the sieve pores about 8-10 in



Pairs of electron stereomicrographs. Figs. 1, 2, *Coscinodiscus Wailesii.* (Portions of a mantle. 1, Inside view. 2, Outside view.) 3, 4, *Rhizosolenia styliformis* var. *latissima.* (3, Portion of a calyptra, showing the depression for a seta. 4, Portion of an intercalary band. 3, 4, Outside views.) Scales: 1 μ.

H. Okuno: Fine structures of diatom frustules



Pairs of electron stereomicrographs. Fig. 1, *Eucampia zoodiacus.* (Outside view.) 2, *Biddulphia sinensis.* (Portion of an intercalary band; inside view.) 3, 4, *Achnanthes longipes.* (3, Portion of an area-valve; inside view. 4, Central portion of a raphe-valve; outside view.) Scales: 1 μ.

$10\,\mu$. A sieve pore, which at the same time represents a shallow secondary loculus, is round or rounded polygonal, about 500–1500 $m\mu$ in diameter, and closed at the top by a netveined membrane supported by several stalks ingrown from the margin of the pore. The meshes of the netveined membrane are round or linear in the center, and curved linear or kidney-shaped at the margin. The pseudoraphe of the area-valve, the axial area and the transverse fascia of the raphe-valve were impenetrable to the electron beam.

Habitat: Marine, littoral. Shirahama, Wakayama Prefecture (Okuno, No. m562. Nov. 1951).

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摘　　要

海産珪藻 6 種類 *Coscinodiscus Wailesii*, *Rhizosolenia styliformis* var. *latissima*. *Chaetoceros affinis*, *Eucampia zoodiacus*, *Biddulphia sinensis*, *Achnanthes longipes* の珪殻につき，それぞれ電子顕微鏡立体写真を撮影し，その微細構造を立体的に解明した。登載した電子顕微鏡立体写真観察用の立体鏡は同一倍率（約2倍位が適当）のルーペ2箇を両眼の距離にはなし，支持枠に固定して作ることができる。（枠で固定せず2箇のルーペを手にもち両眼にあてて立体鏡として使用してもよい。）立体写真を挿図1のように，眼・立体鏡・立体写真の位置を正しく調節して見るとレンズによって拡大された立体像を見ることができる。図版説明文中に inside view または outside view と記したのはこのように両眼の視軸が平行になるように設計された立体鏡を用いた場合の視方向であって，もし立体鏡を，左右の視軸が交叉するように作ると，視方向は前の場合の反対となる。このように1対の立体写真は，その見方を変えるだけで（または写真の左右位置を変えることによって），被写体の内外（裏表）両面よりの立体視を見ることのできる都合のよいものである。また立体鏡を用いず裸眼のままでも立体写真から立体像を見ることができる。すなわち，裸眼のまま1対の立体写真を見つつ，両眼の視線を鼻の方へ少しく寄せると，両写真がそれらの中間で重なり合い立体像を見ることができる。裸眼の場合も両眼の調節変化（両眼の視軸を平行あるいは交叉させる）を工夫練習することによって（または写真の左右位置を反対に変えることによって）1対の立体写真から内外両面観を見ることができる。

Leaf Age and Unfolding Season in the Photosynthetic Activity of Cultivated Mulberry Plants

by Tadayoshi TAZAKI*

田崎忠良*: 栽培グワにおける炭酸同化能力と葉令・開葉期との関係

Received November 27, 1958

Originated from the work of Boysen Jensen[1], the growth analyses of natural plant communities and cultivated fields are in progress by Monsi and Hôgetsu group in our country[2]. The seasonal variation of photosynthetic activity is one of the most cardinal components for the study of growth analysis.

Contributions to this line of study in arboreal plants have been comparatively meager, but recently Kusumoto's[3),4)] and Saeki & Nomoto's[5)] investigations appeared, taking several evergreen and deciduous broad-leaved trees as their materials.

Mulberry plants, in their native life type, belong to deciduous broad-leaved tree and few leaves develop after unfolding season in spring. So the leaf age is almost uniform throughout one individual. Unlike native type, shoots of the previous year are once (in spring or early summer) cut down annually and only old stump near the ground is left in cultivated mulberry plants.

After the shoots were cut down, new shoots grow likewise perennial herbs, that is, stems are elonging and new leaves are unfolding throughout the growing season. So the age and unfolding period of each leaf on one shoot are all different. Cultivated mulberry plants are, as it were, forced artificially to change their life type from tree to herb. The details of cultivation modes will be mentioned afterwards.

In the present paper, the seasonal variation of photosynthetic activity of cultivated mulberry was studied from leaf age and unfolding season by measuring the net assimilation amount of each leaf under light saturated condition and the relations between photosynthetic activity and annual growth were discussed

Material and method

The material used for this experiment was mulberry plants cultivated in the mulberry field of Tokyo University of Agriculture and Technology situated in Koganei, Tokyo. Each plant was spaced 90×180 cm.. The soil was Kantô loam and was manured in the ordinary manner. The pruning method was root training, that is, the stumps were left several centimeters from the soil surface and the shoots sprouted from the

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stumps were annually cut down. There are two methods of harvesting. In "spring cut" mulberry, the shoots of the previous year are totally cut down in early spring before sprouting, and nine new shoots are left to grow, from which leaves are harvested for silk worm rearing in August and September. While in "summer cut" mulberry, the shoots are left intact in spring, from which new lateral shoots develop. After spring harvest of new shoots, the shoots of the previous year are cut down in early summer, and from new shoots developed afterwards leaves are again harvested in August and September. In both harvesting, lower leaves are at first harvested in August and upper leaves in September. In the following experiments, shoots were left intact at summer cutting in some stumps and were left to grow for which it was named "shrub" mulberry by the author* (Fig. 1).

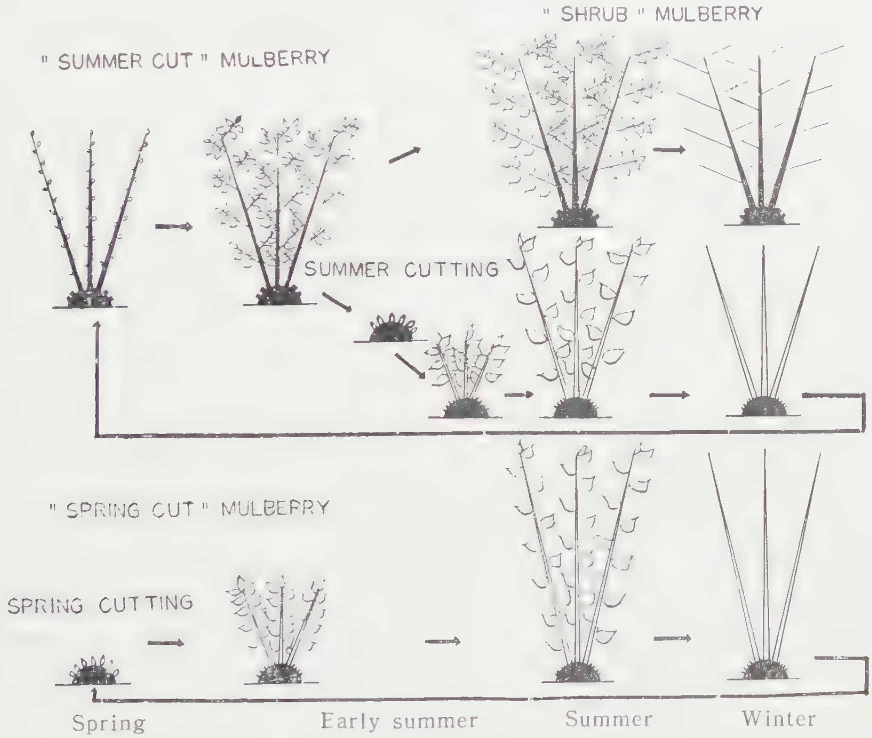

Fig. 1. Two modes of pruning in mulberry plants. Half circle near the soil surface is old stump. Out of nine shoots, six shoots are omitted. Shoots developed in the previous year are pictured black.

All leaves on one shoot were taken as the sample. They were numbered from the older to the younger. As the youngest leaf the author designated those leaves which had finished unfolding and leaf surface was already flat. In another experiment, the youngest leaves were marked biweekly from May 1, and the variation of photosynthetic activity with the aging of leaves was measured. By preliminary ex-

* In practical cultivation, the shoots of the previous year in "summer cut" mulberry are cut down without exception.

periment, it was revealed that the photosynthetic activity of mulberry leaves began to fall in 30 min. from picking even if they were supplied with water and high light intensity. So the measurements were finished as early as possible from picking. On fine days, sunny leaves were sampled from mulberry trees between 9 a.m. and early afternoon, and were supplied with water by a small glass bulb. The assimilatory chamber was immersed in a water bath about 10 cm. deep, and air temperature insides was kept at 23° in summer and about 17°–20° in spring and autumn. The light source was a 300 W flood lamp combined with sky light, and light intensity was determined by Tôshiba No. 5 photometer. Net assimilation and respiration were measured by Boysen Jensen's apparatus a little modified by the author. Stomatal aperture was examined by benzol infiltration and graded in six degrees, from closed (0) to widely open (5). Transpiration amount was measured by detached leaves using a torsion balance, and chlorophyll by the procedure of Kasanaga and Monsi[6].

Result and discussion

The cardinal values in the light-assimilation curve in matured mulberry leaves are as follows: maximum net assimilation 5–7 mg. CO_2/50 cm^2./hr., respiration 0.5–1.0 mg. CO_2/50 cm^2./hr. at 20°, compensation point 0.5–0.7 Klux. These values conform fairly well with the normal leaf of *Zelkowa serrata*[5] and compensation point and respiration are somewhat higher than the sun leaf of evergreen broad-leaf trees[3]. In the vicinity of Tokyo, cultivated mulberry begins to sprout at the middle of April. The day of first unfolding differs by cultivated form and climate of every year. In 1954, the first leaf of Kairyo-nezumigaeshi (a middle ripening form and most commonly cultivated in Kantô district) unfolded on April 21 when two or three leaves in one shoot had already unfolded in early ripening forms such as Nogamisô. The photosynthetic activity of all leaves on the new lateral shoot of "summer cut" mulberry was measured in Kairyo-nezumigaeshi and Nogamisô from the end of April to May, but some fragmentary experiments were done in several other forms. The case of Kairyo-nezumigaeshi was illustrated in Fig. 2. None of the leaves showed positive net assimilation in April under 40 Klux and at 17°. The first positive net assimilation

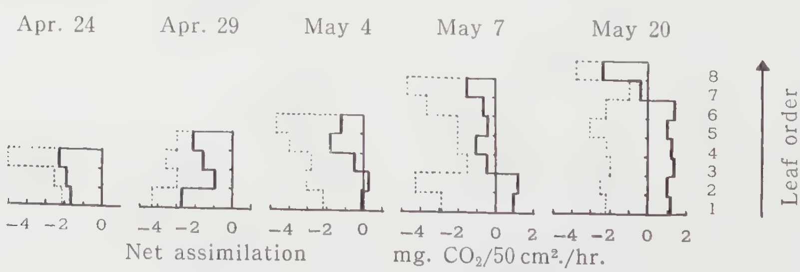

Fig. 2. Net assimilation and respiration of leaves on the new lateral shoot of "summer" cut mulberry (Kairyo-nezumigaeshi) in spring. Solid line indicates the net assimilation under 40 Klux and at 17°, and broken line the respiration at the same temperature. Leaves are numbered from the oldest.

appeared at the beginning of May in lower leaves and most leaves showed positive assimilation on May 20. But the values were quite small in comparison with those of matured leaves mentioned above. Similar trend was seen in Nogamisô and in several other forms. In 1955, the lower leaves on the lateral shoot of Kairyo-nezumigaeshi, Shimanouchi and Ôshimasô showed already positive net assimilation at the end of April, but in both year, net assimilation remained 3 mg. CO_2/50 cm^2. /hr. or thereabout at the end of May, the season of summer cutting. Some yearly fluctuation in net assimilation may exist in cultivated mulberry. Similar trend was obtained in the new shoots sprouted from "spring cut" stumps.

The low values of net assimilation in spring is partly due to the high respiration in this season (See Fig. 2). The chlorophyll content of leaves on the new shoot of "spring cut" mulberry (Kairyo-nezumigaeshi) was measured on May 10, 1957. The chlorophyll content of the 5th, 4th, 3rd, 2nd and 1st leaf was 0.8 0.6 1.5, 2.0 and $2.0 \cdot 10^{-2}$ mg./cm^2., and net assimilation of these leaves was -0.5, 0.6, 1.7, 2.0 and 1.5 mg. CO_2/50 cm^2./hr., respectively. In this case, parallel relation was seen between chlorophyll content and net assimilation. But this relation was somewhat different in Kiba-jyumonji, which genetically has yellow or yellow green leaves in spring or early summer, but the growth is only a little inferior to other ordinary forms. In 1957, chlorophyll content and net assimilation were measured simultaneously. In yellow leaves, the chlorophyll content was below $0.5 \cdot 10^{-2}$mg./cm^2. and the assimilation rate was from -2.0 to 0.2 mg. CO_2/50 cm^2./hr. This low value of net assimilation might be attributed to the low chlorophyll content. In yellow green leaves, however, the chlorophyll content was about $1.0 \cdot 10^{-2}$ mg./cm^2. and the net assimilation about 2.0 mg. CO_2/50 cm^2/hr., so the chlorophyll content was smaller but the net assimilation was equal to or larger than the ordinary forms in this season. The efficient assimilation in Kiba-jyumonji was already affirmed by indirect method[7]. The chlorophyll content in Kairyo-nezumigaeshi and Kiba-jyumonji in this season was smaller than those of matured mulberry leaves, that is, $6 \cdot 10^{-2}$mg. /$cm.^2$. From above results, it may be concluded that high chlorophyll content is not always accompanied with high net assimilation and *vice versa*.

The behavior of stomata in the spring time was examined by measuring transpiration rate and infiltration test. By author's unpublished data concerning the transpiration of mulberry leaves in summer, the stomata open widely in the morning (7 a. m.), remain opened throughout the daytime and close perfectly in the evening after sunset. The relative transpiration was calculated using leaf shaped filterpaper with one side of evaporating surface as an evaporimeter. The value in the day time was often over 100% which is far larger than other deciduous trees.

Compared with Monsi's paper[8)], this value is the same order with *Jussieua* and *Hydrocharis* (water plants) and far larger than various land plants. Out of land plants only *Sambucus* is comparable (95% on Aug. 17). A sprouting shoot of "summer cut" mulberry was cut down and immediately immersed in a 1/200 solution

of purchasable H_2SO_3 (SO_3 6 % in water) in order to ensure the water absorption and to keep the stomatal aperture in the same state of before cutting. As an example, the result in Nogamisô was as follows. The measurement was done in the forenoon of a fine day, April 21, 1954. Four leaves had already developed on the side branch, but the stomata were nearly closed in every leaf. The aperture was 0 or 1. Relative transpiration was around 30 % except in the fourth leaf (21 %). In matured leaves in summer, the relative transpiration when stomata were closed was below 20 %, so the value in spring was somewhat higher which may be due to high cuticular transpiration. The insufficient stomata movement may play some part in the low amount of net assimilation in spring.

In most cultivated forms, the size of matured leaves is too large to measure net assimilation by Boysen Jensen's apparatus. So the measurement was limited only in spring before maturing. In the experiments throughout the growing season, Nezumigaeshi (a small leaf form) was used. In "shrub" mulberry of this form, the youngest leaves of April 29, May 16, May 29, June 15, July 29 and Aug. 15 were marked with colored vinyl tapes and the seasonal variation of net assimilation was investigated in leaves with different unfolding day at saturated illumination of 40 Klux. The results were summarized in Fig. 3. In leaves unfolded in spring (April

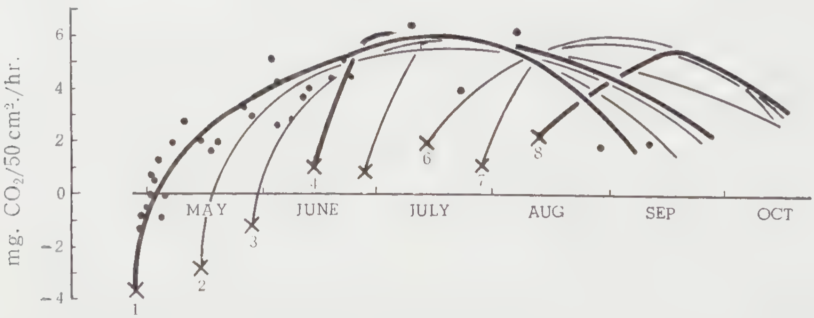

Fig. 3. The seasonal variation of photosynthetic activity in leaves with different unfolding day. The abscissa is the season in month and the ordinate the net assimilation at 17-23° and under 40 Klux. Each curve based on 20-30 measurements. The points were rather scattered and those for only one curve (leaves unfolded on April 29) are given in the figure. The mark x indicates the date of unfolding. The material was "shrub" mulberry of Nezumigaeshi.

29 and May 16), net assimilation showed strong negative value at the day of unfolding, which was quite the same with the youngest leaves of Fig. 2. The net assimilation at first rapidly and then gradually increased during May and June, and attained the maximum value of around 5 mg. CO_2/50 cm^2./hr. at the end of June. This value continued until the middle of August, and then gradually declined. The net assimilation of the unfolding day gradually increased, turned positive from the middle of June. Besides, the net assimilation increased far more rapidly than the leaves in spring, that is, the maximum assimilation appeared only ten days after unfolding.

The maximum value in this case continued until the middle of September and declined in October. The trend of the curve in leaves unfolding before early summer was similar with that of *Zelkowa* in 1953[5]. The duration of maximum activity was about two months, but in leaves developed in summer this duration was much shortened on account of the advent of autumn. Similar experiments were performed in new shoots developed after summer cutting, but no significant difference was seen between those and "shrub" mulberry in the variation of photosynthetic activity with aging of leaves.

Also the seasonol variation of photosynthetic activity was well reflected in the net assimilation measured leaf by leaf in one shoot. The condition of spring was already referred previously (Fig. 2). From the end of May to June, the net assimilation of the youngest leaf gradually increased and finally turned positive, the value increasing from the upper young leaves to the lower older leaves. In this season, shoots are cut down in "summer cut" mulberry. The situation was quite different in the photosynthetic activity of leaves unfolded after summer cutting when compared with those after spring cutting. In 1956, the net assimilation of new shoots (stem +leaf) emerged after summer cutting was measured. From the expanding of buds to the unfolding of the first leaf, the value was negative, that is, about -0.25 mg.

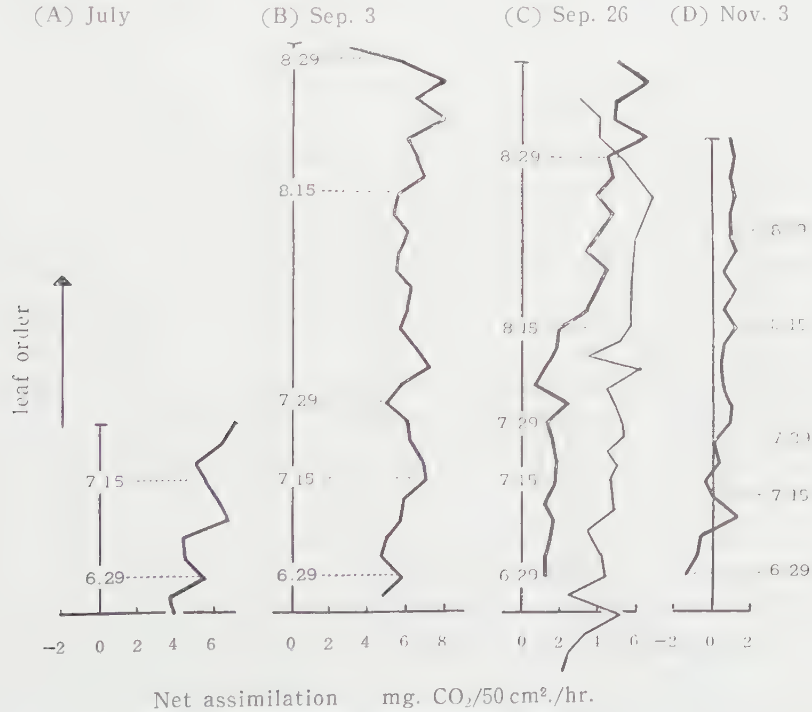

Fig. 4. The variation of net assimilation rate by leaf order in "summer cut" mulberry (Kairyo-nezumigaeshi). Every other leaf was taken for the sample. The date at the top of the figure is the date of measurement and the unfolding date is shown on the ordinates. The thin line in (C) is the net assimilation in "shrub" mulberry on September 20.

CO_2/100 mg. dry weight/hr. The value turned positive as soon as the first leaf unfolded and reached to +1.2 mg. in the same unit when three leaves developed. So the photosynthetic activity was far more efficient in this case than in spring. In summer, nearly all leaves showed maximum net assimilation, for the activity increased rapidly after unfolding in this season and the activity decline by aging of leaves in lower part did not yet set in. Cultivated mulberry, therefore, displays most efficient matter production in this season (Fig. 4 —A and —B).

With the advent of September, net assimilation decreased from lower leaves and next the maximum value was shifted to the uppermost leaf, for the development of leaves gradually stopped in this season. So far, no difference was observed concerning the seasonal variation of photosynthetic activity in three kinds of harvesting methods. But in autumn, the net assimilation of lower leaves decreased earlier in "summer cut" mulberry than in "shrub" mulberry (Fig. 4-C). As the "shrub" mulberry is more natural life form than "summer cut" mulberry, this phenomenon is somewhat interesting. In late autumn just before the defoliation, the photosynthetic activity of upper leaves remarkably diminished and the value turned negative in lower leaves (Fig. 4-D).

From above results, the carbohydrate necessary for the growth of new shoot and for the respiration of non-assimilating organs in spring, it may be conjectured, should mostly be derived from the reserve substance of the previous year, as the photosynthetic activity in this season is too low to fulfill these requirements. Then, whence comes these reserve substances? In "summer cut" mulberry, the dry weight increment of leaves in mid-summer was 48 g. for one shoot during 40 days. So the daily increment was 1.2 g.. Assuming that the net assimilation is 5 mg. $CO_2/50\ cm^2$./hr., nocturnal respiration 0.5 mg. in the same unit and leaves assimilate in this rate for 13 hours, one shoot assimilates 3.6 g CO_2 or 2.5 g. dry weight per day. Out of 2.5 g. of assimilate, 1.2 g. must be used for the growth of this shoot and the rest may be consumed for the growth of roots and stump, and for the respiration of non-assimilating organs. So in summer there may be no room for accumulating reserve substances. At the end of September, the growth stops and from this season, the assimilates may be stored for the growth of the next year. Considering the low net assimilation of lower leaves, the mean net assimilation of "summer cut" mulberry in autumn may be estimated 3 mg. $CO_2/50\ cm^2$./hr. and respiration 0.5 mg. in the same unit. If we assume that the leaves assimilate in this rate for 10 hours, 1 m^2. of leaves assimilate 4.6 g. CO_2/m^2/day or 3.1 g. dry matter. The dry weight of 1 m^2. of mulberry leaves was 60 g.. So the leaf must work 20 days to reproduce itself in the next year. In fact. "summer cut" mulberry produce larger amount of leaves till the end of May (before summer cutting) in the next year. Besides the growth and respiration material of non-assimilating organs must depend upon the assimilate in autumn.

For this reason, it may be concluded that the assimilation in autumn has much

to do with the growth of the next year. In sericultural practice, upper leaves are often taken for silk worm rearing in autumn, which often cause serious influences on the spring yield of mulbery leaf in the next year.

In "summer cut" mulberry, total assimilating organs are cut down after exhausting reserve substances for vernal growth which may cause drastic blow to the physiology of mulberry plant. Even before cutting down, reserve starch disappeared in every part of the plants, and reappeared at the end of August, than is, three months after cutting down. The leaves are smaller and somewhat thinner than those of "spring cut" mulberry. The leaf yield in autumn is only the half of the "spring cut" mulberry. As mentioned above, the duration of maximum photosynthetic activity is far shorter and the anomalism in water physiology, that is, the inert movement of stomata in water active reaction comes earlier than other culture modes. In every aspect, disadvantageous symptoms for life were observed in "summer cut" mulberry.

Summary

The seasonal variaton of photosynthetic activity of cultured mulberry plants in three modes of culture, that is, "spring cut," "summer cut" and "shrub" mulberries, was studied from the viewpoint of leaf age and unfolding season.

1. The cardinal values in the light-assimilation curve in matured mulberry leaves were as follows: Maximum assimilation 5-7mg. CO_2/50cm.^{2}/hr., respiration 0.5 -1.0mg. CO_2/50cm.^{2}/hr., and compensation point 0.5-0.7 Klux.

2. The leaves unfolded in spring showed at first strong negative net assimilation, and the first positive value appeared from the end of April to the beginning of May. This low rate of activity may be due to the high amount of respiration, low chlorophyll content and inadequate stomatal movement.

3. The net assimilation which showed strong negative value on the day of unfolding, at first rapidly and then gradually increased during May and June, attained the maximum value of around 5 mg. CO_2/50 cm.^{2}/hr. at the middle of June. This maximum activity continued for two months till the middle of August. The activity at the unfolding day gradually increased as the progress of season and turned positive from the middle of June. Besides, the net assimilation increased far more rapidly in the leaves unfolded in later seasons than in those developed in spring, that is, the maximum assimilation appeared only ten days after unfolding.

4. The seasonal variation of photosynthetic activity was well reflected in the net assimilation measured leaf by leaf in one shoot. From April to June, the net assimilation increased from the upper to the lower leaves. In summer, nearly all leaves showed maximum assimilation, for the activity increased rapidly after unfolding in this season. With the advent of autumn, net assimilation decreased from the lower leaves and next the maximum value was shifted to the uppermost leaf as the

development of leaves stopped in this season. In late autumn, the activity of all leaves remarkably diminished.

5. From above results, the carbohydrate necessary for the growth of new shoots in spring must depend upon the assimilates of the previous year. As the assimilates in summer were used chiefly for the growth, the assimilates of autumn will fulfill this requirement.

6. In "summer cut" mulberries, total assimilating organs are cut down after exhausting reserve substance for vernal growth, which must be a drastic blow to the physiology of mulberry plants. In fact, the duration of photosynthetic activity was shortened and the anomalism in water physiology appeared. The size and yield of leaves were much reduced in comparison to those of "spring cut" mulberry.

The author wishes to express his sincere thanks to Prof. M. Monsi and Prof. K. Hôgetsu for their valuable advice throughout the progress of this study, and thanks are also due to Messrs. H. Abe and S. Teshigawara who helped the experiments.

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摘　　要

栽培クワの葉令・開葉期と炭酸同化能力の変化を調べた。成熟葉の光-同化曲線は一般の広葉樹とおなじである。春の開葉期には光飽和下でも純同化量はマイナスであり，4月下旬から5月上旬にかけてプラスに転ずる。

純同化量が小さい原因としては，呼吸量が大きいこと，クロロフィルが少ないこと，気孔の運動が活発でないことなどが考えられる。

春に開いた葉の純同化量は季節の進むにつれて増大し，6月中旬には最大値に達し，8月下旬にはふたたび減少する。初夏からあとに開いた葉では，開葉時の純同化量は大きく，すでにプラスであり，その後すぐ最大値に達する。

葉位に伴なう純同化量の変化には，葉令の関係がよく反映されている。春から初夏にかけては，下部の古い葉ほど純同化量が大きいが，夏には葉位による変化は少なく，すべての葉の純同化量は大きい。秋には下部の葉から小さくなり，落葉前には上部の葉におよぶ。クワの生理から見ていちばん不自然な夏切りグワでは，秋に純同化量がはやく落ち，また生長もわるく葉も小さく，気孔の水能動反応もほかのしたてかたをした時よりはやく活発でなくなる。

On Four New Halophilic Species of *Spirillum*

by Narumi WATANABE*

渡辺成美*： 好塩性螺旋菌 4 新種について

Received October 11, 1958

Though the genus *Spirillum* Ehrenberg was not so intensively investigated as other groups of bacteria were, Giesberger[1] (1936) has achieved an extensive work on *Spirillum*. Myer[2] (1940) isolated four spirilla with the single spore technique described by Kauffman, using beef-peptone broth. Cayton and Preston[3] (1955) also isolated a new species of *Spirillum*, *S. manucuniense*.

All these species reported[4] were, however, found in fresh water and no isolation from salt water has been ever reported so far as the writer knows. This fact seems indicate that the *Spirillum* in salt water might hardly grow on the media usually available.

The writer[5] has succeeded to isolate halophilic spirilla from visceral organs (mainly in alimentary tracts) of marine shell-fishes; *Venerupsis philippinarum*, *Mactra veneriformis*, *Meretrix meretrix*. In this paper an isolation technique which is comparatively simple and effective, and a description of four halophilic spirilla newly obtained from the shell-fishes will be reported.

Methods

Medium: The first step of the culture is the search for a solid medium on which it may grow. Kutscher (1895), Vogt (1899), Van Iterson (1902), Beijerinck (1925), Dimitroff (1926) and others isolated the fresh water spirilla using the peptone-solution or the medium containing peptone, and Giesberger cultured the same spirilla with the following synthetic medium: 0.2—1 % Calcium lactate, 0.1 % NH_4Cl, 0.05 % K_2HPO_4, 0.05 % $MgSO_4$.

Regarding the *Spirillum* in sea water, it should be natural to assume sodium chloride and the other ingredients of sea-water as elements of the medium. Hence, a mixture of the organic and inorganic substances described above will be appropriate for the medium of halophilic bacteria. From the above-stated reason, Medium No. 1, devised by the writer, is appropriate for enrichment culture, and Medium No. 2 for isolation.

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No. 1

NaCl	25 g.
$MgCl_2$	3
$MgSO_4$	1
$CaSO_4$	1
K_2SO_4	1
$CaCO_3$	0.5
Peptone	2-5
Water	1000 ml.

(pH, 7.0-7.2)

No. 2

NaCl	25 g.
Calcium lactate	10
NH_4Cl	1
K_2HPO_4	0.5
$MgSO_4$	0.5
Peptone	2-5
Water	1000 ml.

(pH, 7.0-7.2)

When pure isolate was obtained, one of the two may be used for subculture. In these media the specially essential components are NaCl and peptone. The amount of peptone in the medium must be at least 0.1 % at the adequate concentration of NaCl, i. e. 2 to 2.5 %. If the concentration of NaCl in medium becomes lower, the spirilla can not normally develop. They actually grew poorly in this medium in the shape of rod or dott after several days. And they gradually sank to the bottom of the test tube. Of course, the repeated subculture is impossible in the medium without NaCl. In the experiments for identification, the writer used the bouillon, peptone-water and others, which contain 2.5 % NaCl, besides No. 1 and 2.

Isolation: In this experiment, three shell-fishes were used; *Venerupsis philippinarum, Mactra veneriformis* and *Meretrix meretrix,* collected at Samugawa beach in Chiba city. Following procedures were taken for isolation of the bacteria.

1) After washing with water, several living shell-fishes are put into a Petri dish containing 2 % NaCl solution.

2) The Petri dish is left in the room. The shell-fishes die and open the shell in a few days. When putrefaction occurs conspicuously, some kinds of spirilla are usually detected microscopically in the liquid.

3) To obtain an enrichment culture of the bacteria, this liquid is inoculated to Medium No. 1, in which spirilla grows abundantly after a week at 20°–23°. Instead of Medium No. 1, we can use the marine water containing 1 % peptone.

4) If we streak the bacteria on the plate using Medium No. 2 added with 1–1.5 % agar, the peculiar and coarse granular colonies, on which Ca-crystals develop, are found with the naked eye after 4 or 5 days.

Descriptions of the species

Spirillum japonicum nov. sp.

I Morphological aspect.

1) Vegetative cells: Stout threads with several or many wave-like undulations. 1.2 to 1.5 microns in diameter. Wave length is 15 to 18 microns. Width of spiral, 4 to 5 microns. Seldom total length about 50 microns. Rounded end. Solitary. Gently motile. Tufts of 20 to 30 flagella at each pole. Flagella move in unison. Cyst-like body is formed. Volutin granules present in cytoplasm. Gram negative.

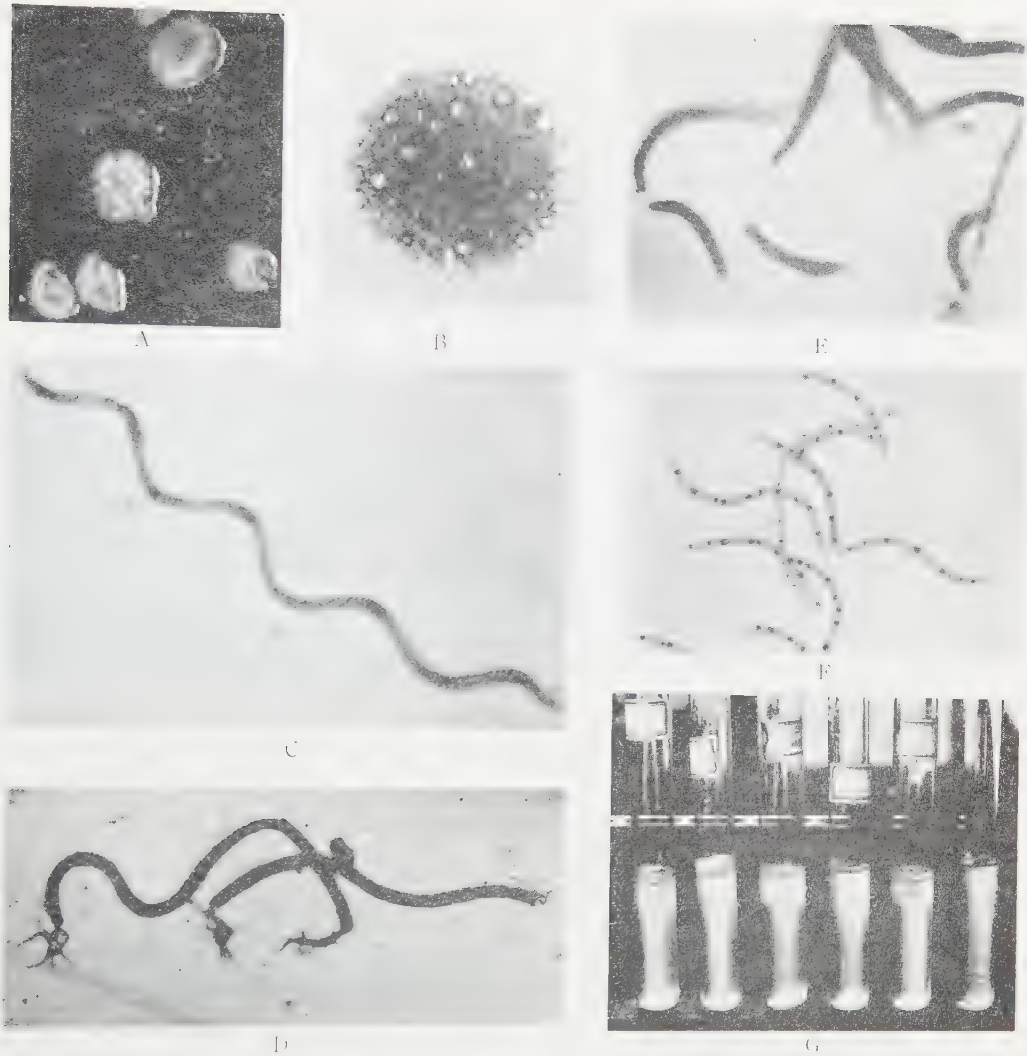

Fig. 1. *Spirillum japonicum* nov. sp.

A. Colonies on Medium No. 2 agar. Ca-crystals appear like oil droplets on the surface of the colony. ×10

B. Enlarged form of the colony. After 10 days. Small Ca-crystals are scattered on the surface. ×30

C. Typical vegetative cell stained with dilute Löffler's methylene blue as twice (vital staining method). On Medium No. 1. ×1350

D. Flagella-staining by Nishizawa & Sugahara method. ×1350

E. Vegetative cells obtained from colony. Not spiral form. ×1350

F. Volutin granules appear in cytoplasm in old stage. ×1100

G. Mass-culture as layer of 1-1.5 cm. in liquid.

II Cultural aspect.

2) Agar colonies: Heavy growth on Medium No. 2 (A)*. Colonies are seen

* (A) indicate Medium No. 2, and, (B) bouillon.

with the naked eye after 4-5 days. Normal growth on bouillon agar (B)*. Round, entire somewhat undulate contour. Punctiform, (A) 0.5 mm. in diameter and (B) about 0.8 mm.. Pulvinate, coarsely granular or moruloid. Surface smooth, (A) but with small Ca-crystals scattered at first and as a scab later. White to lactescent, (B) pale yellowish brown colour like bouillon agar. Semitransparent.

3) Individual form on solid medium: Rod, curved or gentle wave. 1 micron or less in diameter, seldom 30 to 35 microns in length.

4) Agar stroke: Poor development, filiform or beaded, flat, glistening luster. Surface smooth, (A) formation of scab. White colour. (B) Colour of deposit in the condense water is lactescent to pale yellowish brown. (A) Cheese-like, (B) Slimy.

5) Agar stab: Development on surface, but not inside. Barely filiform. No colouration of medium.

6) No gelatin liquefaction. On bouillon gelatin containing 2.5 % NaCl and Medium No. 2 gelatin, colonies do not appear.

7) Liquid media: (A) Ca-crystals as a membrane on surface of medium or as a ring along the tube wall. (B) No change. (A) Intense turbid at upper portion as thick layer (about 1 to 1.5 cm.). (B) Clouded; clouded masses precipitate by slight stimulus. White slimy compact deposit, a large quantity. No bad odour.

III Physiological aspect.

8) Tolerance for concentration of NaCl: Minimum point 1 %. Optimum point 2.5 %. Tolerable for 5.5 to 6 % NaCl.

9) Indole not formed. 10) Aerobic. 11) Optimum temperature 18° to 20°. Max. temp. 30°. 12) Potato: White colour. 13) No reduction of nitrates and nitrites. 14) Catalase negative. 15) No dissolution of saccharides. 16) Litmus milk: Unchanged. Litmus in liquid media: No change. 17) pH: No development below pH 6.6. When growth of bacteria ceases in liquid, pH of the medium becomes 8.2.

Spirillum halophilum nov. sp.

I Morphological aspect.

1) Vegetative cells: Slender forms with one to three (seldom several) wave-like undulations. 0.5 to 0.6 microns in diameter. Wave length, 3.5 to 4 microns. Spiral width, 1.2 to 1.5 microns. Motile with bipolar tufts of flagella (5-6). Form of flagellum is wave-like. Wave length, 4 microns. Length, 7 to 8 microns. Wave amplitude, 1 micron. Volutin granules present at old stages. Gram negative.

II Cultural aspect.

2) Agar colonies: (A) Growth not speedy, (B) normal growth. Round, entire, circular (1-1.5 mm. in diameter). Convex to capitate, finely granular, coarse grains in centre. Smooth surface. (A) Somewhat large Ca-crystals as a scab. Grayish white colour. (B) Yellowish like bouillon. Semitransparent.

3) Individual form obtained from colony: Spiral, 5-6 cycles. Spiral width 1 micron, wave length 4-5 microns.

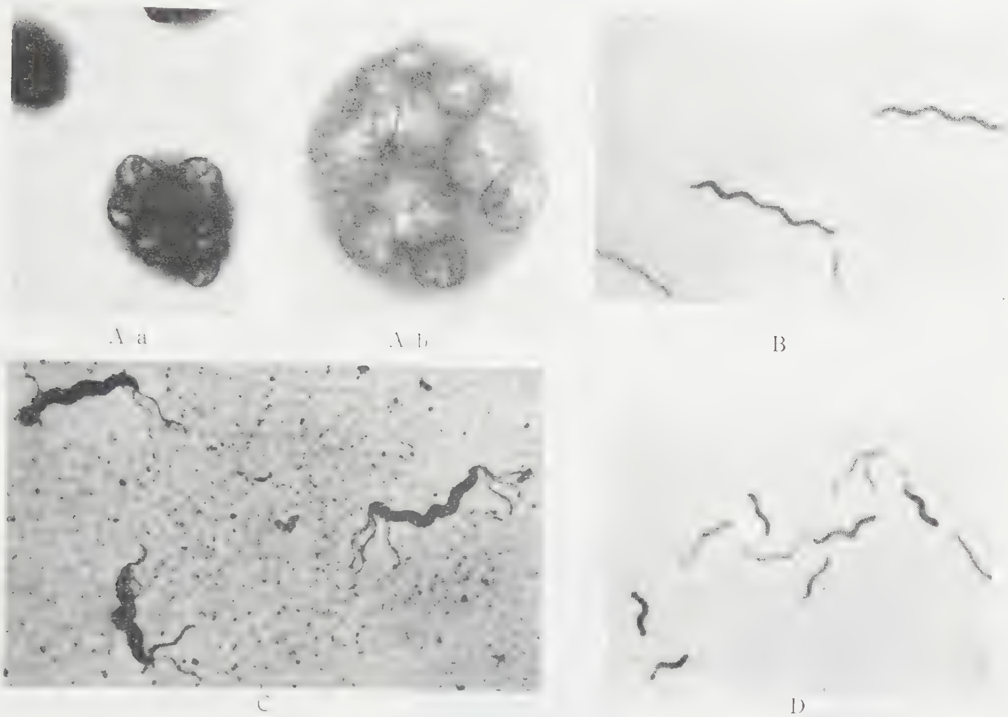

Fig. 2 *Spirillum halophilum* nov. sp.

A. Colony on No. 2 agar. Somewhat large Ca-crytals are crowded like a scab. (By taking the focus on centre point, the margin of the colony does not appear in photograph.) A a. ×20. A-b. Form of Ca-crystals. After a week. ×30

B. Normal vegetative cells by vital staining of dilute Löffler's methylene blue. (On Medium No. 1) ×1350

C. Flagella-staining. ×1350

D. Vegetative cells obtained from the colony. ×1350

4) Agar stroke: Moderate development, filiform, flat, glistening, smooth surface at margin but scab at centre. Grayish yellow colour on peptone agar, white on Medium No. 2. Colour of deposits in the condense water is pinkish. Slimy dense deposits.

5) Agar stab: Development on surface, not inside. Barely filiform. Surface colour: Brownish orange at first, later brown. Medium becomes grayish-pink.

6) No gelatin liquefaction.

7) Liquid media: No change, Ca-membrane is barely produced at old stage. (A) Moderate turbid, diffused. (B) Especially at upper portion. Clouded masses precipitate by slight stimulus. No bad odour. Slimy clouded deposit. Amount of deposit is proportional to cultural period. Medium becomes yellow.

III Physiological aspect.

8) Tolerance to concentration of NaCl: Minimum point 1.5 %. Optimum point 2.5 to 3 %. Maximum point may be 6 %.

9) Indole not formed. 10) Aerobic. 11) Optimum temperature 20°. Maximum temperature 30°. 12) Potato: Pale brown with somewhat pink. Poor growth. 13) No reduction of nitrates and nitrites. 14) Catalase negative. 15) Little acid from glucose. No acid from other saccharides. 16) Litmus milk: Unchanged. Litmus in liquid media: No change. 17) Optimum pH: 6.8–7.4. When growth of bacteria ceases in liquid medium (about 10 days after), pH of medium becomes 8.0.

Spirillum maritimum nov. sp.

I Morphological aspect.

1) Vegetative cells: Spirals consisting of $\frac{1}{2}$ to $1\frac{1}{2}$ complete turns. Diameter, 0.6 to 0.7 microns. Wave length, 4 to 5 microns. Width of spirals, 2 microns. Slight attenuated end. Active motile. A single flagellum at both ends. Length of flagellum 8–10 microns, almost two waves. Formation of cyst-like body is conspicuous. Volutin granules present. Gram negative.

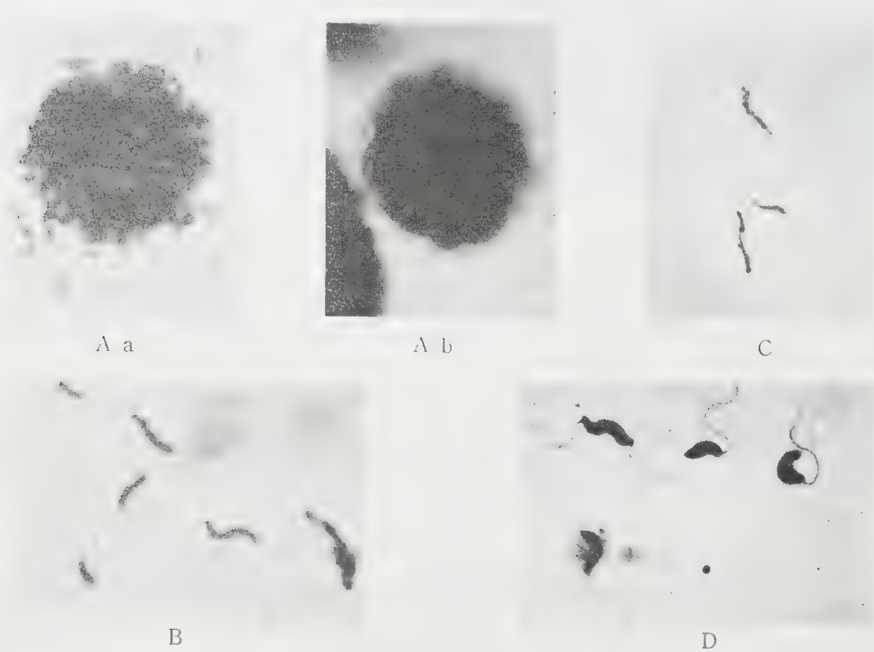

Fig. 3. *Spirillum maritimum* nov. sp.

A. Colony on No. 2 agar. A–a. Scattering small crystals on and around the colony. ×30 A-b. Small crystals appear like a scab in cluster. ×30

B. Normal vegetative cells by vital staining. (On Medium No. 1) ×1350

C. Old vegetative cells with volutin granules. ×1100

D. Flagella-staining treated with Nishizawa & Sugahara I solution and carbolfuchsin as II solution. ×1350

II Cultural aspect.

2) Agar colonies: Slow growth, round, entire, punctiform (0.5–1 mm. in diameter). Pulvinate or convex. Finely granular. Surface smooth. (A) Production of small crowded Ca-crystals as a scab or scattered Ca-crystals on and around the colonies.

Lactescent. (B) Yellowish white colour. Semitransparent.

3) Individual form obtained from colony: Half to one spiral. Wave length 3–3.5 microns. Spiral width 1.5 to 1.7 microns.

4) Agar stroke: Very poor development, filiform or beaded on bouillon and peptone agar, flat, glistening. Surface: smooth and scab. Faint yellow on peptone agar. (A) White colour. Slimy density.

5) Agar stab: No growth along straight needle or barely filiform. No colouration of medium.

6) No gelatin liquefaction.

7) Liquid media: (A) Ca-crystals as a membrane on the surface of medium and as a ring along the tube wall. (B) No change. (A) Intense turbid at upper portion about 1 cm. as layer. (B) Clouded the same as. Slimy clouded deposit, a large quantity.

III Physiological aspect.

8) Tolerance for NaCl: Minimum point 1 %. Optimum point 2.5–3 %. Maximum point 6 %. 9) Indole not formed. 10) Optimum temperature 18°–22°. 12) Potato: Pale white colour. 13) Nitrates, nitrites, no reduction. 14) Catalase negative. 15) Acid from glucose and mannose. No gas. 16) Litmus milk: No change, no coagulation. 17) Optimum pH 6.8–7.2. When growth of bacteria ceases, the pH of medium becomes 8.2–8.4.

Spirillum minutulum nov. sp.

I Morphological aspect.

1) Vegetative cells: The smallest of the spirilla. Spirals consisting of one complete turn in young stage, old stage showing several turns. 0.2–0.3 microns in diameter, spiral amplitude 1.2 microns, spiral period 3.2–3.3 microns. Free. Motile with a single flagellum at one or seldom both ends in young. Amphitrichic (3–4) in old. Undulate flagella, total length 7.5 microns, wave length 2.5 microns, wave width 1.3–1.5 microns. Volutin granules may be present. Gram negative.

II Cultural aspect.

2) Agar colonies: (A) Very slow growth. (To see the colonies with the naked eye, it needs 5–6 days.) (B) Slow growth. Round, irregular lobate. (A) punctiform (0.5 mm. after a week), (B) circular (1–1.5 mm.). Convex. Amorphous. Smooth surface, granular in advanced stage. (A) Milky white. (B) Pale yellow. Semitransparent.

3) Individual form on solid medium: One to two waves. Spiral period 3 microns. Spiral width 1.5 microns in maximum.

4) Agar stroke: Moderate to poor growth, filiform, convex or flat, glistening. Surface, more or less scab at centre but smooth at margin. Faint grayish yellow on peptone agar. (A) White colour. Slimy density.

5) Agar stab: Development well at surface but not inside, barely filiform. Colouration of medium, no or pale brownish yellow.

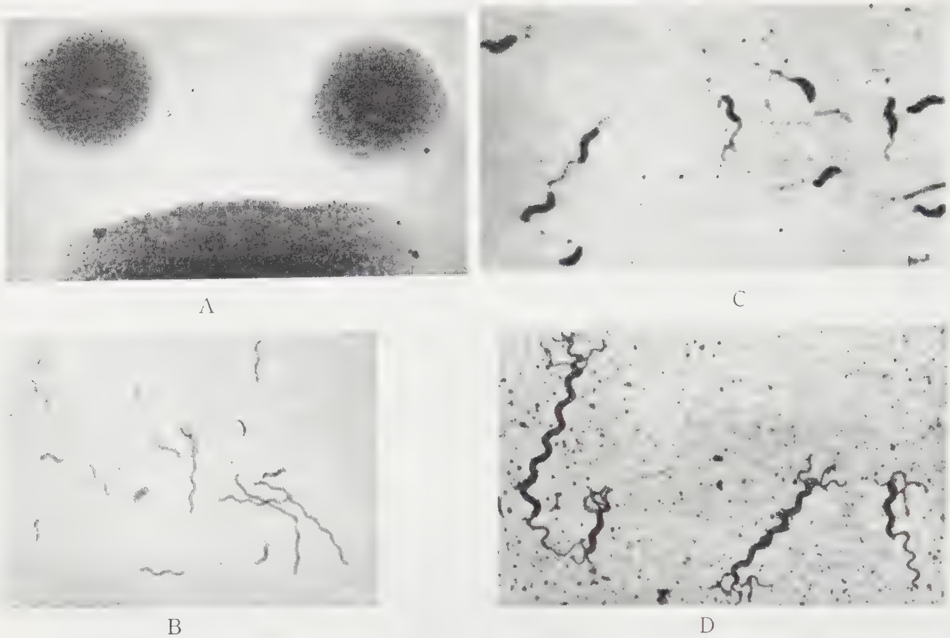

Fig. 4. *Spirillum minutulum* nov. sp.

A. Colonies on No. 2 agar. After a week. ×30
B. Normal vegetative cells by vital staining. ×1350
C. Young vegetative cells treated with flagella-staining. A single flagellum at each pole. ×1350
D. Old vegetative cells with tufts of flagella at each pole. ×1350

6) No gelatin liquefaction.

7) Liquid media: No change. Moderate turbid and diffuse. Slimy flocculent deposit, a large quantity. In bouillon and peptone-water with 2.5 % NaCl, this bacterium luxuriates well and media become turbid.

III Physiological aspect

8) Tolerance for NaCl in medium: Minimum point 1 %. Optimum point 2–3 %. Tolerable for 5.5 %. 9) Indole not formed. 10) Facultative aerobic. 11) Optimum temperature 15°–22°. Maximum temperature 30°. 12) Potato: Scanty development, grayish. 13) Reduction of nitrates: Positive. Reduction of nitrites: Negative. 14) Catalase negative. 15) No acid and no gas from saccharides. No H_2S. 16) Litmus fades on liquid media with maltose or saccharose. Litmus milk, unchanged. No coagulation. 17) Optimum pH 6.8–7.4. 18) Source: Widely distributed in the viscera of marine shell-fishes.

Discussion

If these spirilla in question isolated from the visceral organs of marine shell-fishes are judged by comparison with the species[7, a)] described by the previous investigators from a morphological point of view, *Spirillum japonicum* bears resemblance to

Spirillum volutans—the giant spirillum, which can not be cultivated on artificial medium till now in spite of the efforts of many investigators. *Spirillum halophilum* has resemblance to *Spirillum itersonii*[1] and *Spirillum maritimum* to *Spirillum virginianum*[6]. But it is difficult to compare these halophilic spirilla physiologically with the fresh water spirilla, because these spirilla in question need conditionally the media added with 2 to 2.5 % NaCl on the occasion of isolation and repeated subculture. Therefore, it is assumed that their ability to tolerate NaCl-grade is the specific character given to spirilla in question. In addition to this fact, it is remarkable that the thematic halophilic spirilla must be cultivated by lower temperature than the already known spirilla.

In Bergey's manual[7,b] reports of following three uncertain marine spirilla are cited.

(1) *Spirillum ostreae*.[8] Judging from the original description by Noguchi, it is estimated that this species rather belongs morphologically to genus *Spirochaete* than genus *Spirillum*. This species was named without cultural studies.

(2) *Spirillum attenuatum*.[9] This species has also as much incomplete description as the previous one. Ford states that this organism would be regarded either as a *Spirillum* or as a *Spirochaete*.

(3) *Spirillum colossus*.[10] H. Fischer said that this giantest species of all spiral bacteria is perhaps identical to *Spirillum volutans* var. *robustrum*.

Because there are no physiological as well as morphological studies on these three species, the writer can not examine and compare the halophilic spirilla isolated here from marine shell-fishes with the spiral bacteria reported before.

Summary

Four halophilic spirilla were isolated from the marine shell-fishes: *Venerupsis philippinarum*, *Mactra veneriformis* and *Meretrix meretrix*, which were collected at Samugawa beach in Chiba city. After the morphological and the cultural aspects each of them was diagnosed as a new species and was given respectively following name.

Spirillum japonicum
Spirillum halophilum
Spirillum maritimum
Spirillum minutulum

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摘　要

螺旋菌 (*Spirillum*) の研究は，従来，淡水産種についてのみ行なわれており，海水産種についての知見はまだない。前者についても，その分離培養は一般に困難とされ，現在まで正確な種として記載されたものは 10 種にすぎない。筆者は千葉市寒川海岸産の二枚貝，アサリ，ハマグリ，シオフキ等の内臓内に，好塩性螺旋菌が生育していることを確認した。本菌の培養を集積培養と分離培養とに区別し，前者には海水成分要素にペプトンを添加した培地を，後者には食塩・ペプトン・乳酸カルシウム等を主成分とした培地を使用した。二枚貝の腐敗浸出液より，有効的な平板分離法により純粋培養にみちびいた。

分離された螺旋菌 4 種は，形態的所見のほかに，培養的に生理学的に検討し，本菌が (1) 分離用固上で特異な集落を形成すること。(2) きわめて好塩性で，高い食塩濃度に耐性を有するとともに，海水と同程度の食塩濃度培地に良好な発育を示し，食塩無含有培地上では発育が絶無であること。(3) 淡水産の既知種より低温度において正常発育を示す事実を認めた。以上によって問題の菌 4 種は，淡水産種から明らかに区別さるべきものと認定し，従来正確な記載なしに海水産螺旋菌として命名された種と比較研究した。この結果，ここに好塩性螺旋菌 4 種を新種として同定し記載した。

On the Ploidy of *Torula rubra* Saito*

by Shoitiro IGUTI**

井口昌一郎**: *Torula rubra* の倍数性について

Received November, 19, 1958

Torula has no complete sexual cycle; and though it has been referred to as a stable haplobiont derived from a *Saccharomyces* yeast[1),2)], Schultz and Pomper[3)] have questioned this inference, on the ground of their finding that *Torula* species utilize a wider range of amino acids than do the haploid *Saccharomyces* yeasts. As for discrimination between the haploidy and the diploidy of the *Torula* species, however, the cytological situation has not yet been satisfactorily elucidated, nor is genetical evidence available. The writer's attempt to induce sporulation on the various sporulation media devised by Lindegren[1)] has so far been unsuccessful; moreover, application of the mass-mating technique still failed to cause copulation of *T. rubra* with the haploid *Saccharomyces* of standard mating type *a* or α.

In this respect, analysis of survival curves on a multitarget basis[4)] seems to be quite useful: since diploid involves the genetic units in duplicate, a plot of log fraction survival against dose of irradiation falls onto a straight line, which extrapolates back to about 2, while in the case of haploid it passes through 1[5)]. Herewith, an effort has been made to analyze the survival curve of *T. rubra* under ultraviolet irradiation. The present paper includes the results of the investigation on this species, and also of those made on both haploid and diploid strains of *S. cerevisiae* as serving as the basis of comparison.

Material and Method

Yeast strains. The stock of *T. rubra* used was originally obtained from the Nagao Institute in Tokyo several years ago, and it has been stocked in this labolatory since then. For the parallel experiments, were employed the stocks of *S. cerevisiae* as material, i. e., the haploid strain, *8256*, which requires adenine for its growth, and the diploid strain, *MD-11*, obtained by H. Saito (1956) through mating the two Lindegren's haploid stocks, *8256* (mating type *a*) and *17807* (mating type α).

Media. Constituents of the three synthetic media adopted for the present study are as follows:

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* Contribution from the Biological Institute of Ibaraki University, No. 32.

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(1) Complete medium

Glucose	20.0 g.
Peptone	3.5 ,,
KH_2PO_4	2.0 ,,
$MgSO_4$	1.0 ,,
Yeast extract	1.0 ,,
Thiamin	200 γ
Riboflavine	200 ,,
Ca-pantothenate	200 ,,
Nicotinic acid	200 ,,
Nicotinic amid	200 ,,
Biotin	2 ,,
Folic acid	100 ,,
Inositol	0.01 g.
*Adenine	0.01 ,,
Distilled water	1 000 ml.

* Omitted from the media for *MD 11* and *T. rubra*.

(2) Minimal medium

Glucose	20.0 g.
Asparagine	2.0 ,,
KH_2PO_4	1.5 ,,
$MgSO_4$	0.5 ,,
$CaCl_2$	0.33 ,,
$(NH_4)_2SO_4$	2.0 ,,
KI	0.01 ,,
Ca-pantothenate	200 γ
Biotin	2 ,,
*Adenine	0.03 g.
Trace element solution	Burkholder's prescription
Distilled water	1,000 ml.

(3) Nitrogen-free medium: identical with the complete medium but without peptone.

Procedures. A small number of the cells from the stock culture were incubated on the slant of the complete medium for 3 days at 27°. The fresh cells from the slant were then heavily inoculated on the slant of the nitrogen-free medium and once again incubated 2 or 3 additional days at the same temperature. After this incubation three loops, each 2 mm. in diameter, of the cells were suspended in 5 ml. of distilled water, shaken 10 minutes by a vibrator, and passed through a glass filter (3G–3 of Shibata) to remove any budding or clumping cells from the sample. One ml. of the filtrate was re-suspended in 2 ml. of 10 per cent gelatin solution kept at 37°, and the solution contained 4×10^6 cells per ml.. With a loop of 2×4 mm. in size, film of gelatin was taken up from the suspension and dried for 10 minutes in a horizontal position. The film sufficiently solidified was then exposed for appropriate lengths of time to ultraviolet rays of 2618 Å in wavelength, the rays being served from a Cu-arc light passing through a quartz monochrometer[6]. Immediately after the exposure, the loop was dipped into 1 ml. of sterile distilled water at 37°, the gelatin film being melted therein. Each 0.1 ml. of this water suspension containing 4 or 5×10^3 cells per ml. was spread on each of four agar plates; the irradiated samples of the haploid *Saccharomyces 8256* were plated both on the complete and on the minimal medium two plates each: those of the diploid *Saccharomyces MD-11* and *T. rubra* stocks were plated on the complete medium exclusively. Three or four samples from each stock were irradiated and treated in this way. After a 3 day incubation at 27° in the dark, fraction survival was determined for each plate; the results were then averaged for each exposure for each stock.

Results and Conclusions

Survival curves are shown in Fig. 1, in which the log fraction survivals are plotted as against the irradiation dose in minutes. The curve for the diploid

population (*MD-11*) shows a shoulder phase leading into a logarithmic inactivation phase at about 50 per cent survival, the latter phase extrapolating to about 2. In contrast, the haploid populations (*8256*) exhibit exponential survival curves extrapolating to 1, regardless of the kinds of media, the complete or the minimal. *T. rubra* population is inactivated also exponentially, and the curve clearly indicates an extrapolate at 1. This suggests, contrary to Schultz and Pomper[3], this species to be haploid, even though it lacks both mating and sporulation abilities. The inactivation curves of the haploid *Saccharomyces* and *T. rubra* have commonly resistant phase initiating at about 10 or 15 per cent survival. But it is not certain whether this phase depends upon the resistant mutants induced by irradiation or upon the cells sheltered from the exposure in the back of the wire loop. No determination was made beyond 120 minute irradiation, because it was impossible to get a continuous spark over this time limit by the aparatus here used. After irradiation the inactivation rate of the haploid cultures seems to differ one another, according to the different media; it is more rapid in the minimal than in the complete medium. This difference may, at least in part, be due to the occurrence of a certain mutant or mutants, which cannot grow on the minimal medium. Definite conclusions, however, must await further investigation.

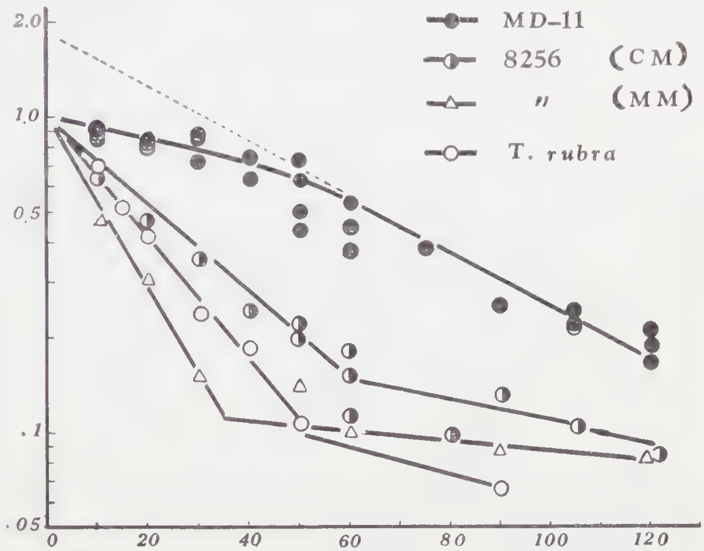

Fig. 1. The survival curves of *T. rubra*, and the haploid and diploid strains of *S. cerevisiae*. Ordinates: survival fraction; abscissae: dose of ultraviolet in minutes. CM: complete medium; MM: minimal medium.

Summary

The survival curve of *Torula rubra* irradiated by ultraviolet rays of 2618 Å was investigated, in comparison with those of both the haploid and diploid strains of *Saccharomyces cerevisiae*. The population of *T. rubra* and that of the haploid *Saccharomyces* are inactivated exponentially, and the curves extrapolate to 1, while the survival curve of the diploid *Saccharomyces* has an extrapolate at about 2. On the basis of the multitarget hypothesis, *T. rubra* is tentatively determined to be haploid. The irradiated haploid *Saccharomyces* is inactivated more rapidly in the

minimal than in the complete medium.

Acknowledgment

The writer wishes to express his sincere gratitude to Professor Y. Yamaguti, under whose generous supervision the present work was carried out; to Professors B. Wada of Tokyo University and F. J. Ryan of Columbia University for their kind interest and suggestions; to Mr. H. Saito of the Institute of Applied Microbiology of Tokyo University for helpful advice and kind supply of haploid (*8256*) and diploid (*MD-11*) strains of *Saccharomyces cerevisiae*. Also to Professor Y. Watanabe of Toho University acknowledgement is due for valuable assistance in preparation of the manuscript.

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摘要

酵母菌の染色体数は確定していないので，それが胞子をつくるか，または接合するかにより，それぞれ二倍体または半数体ときめられている。それゆえ胞子もつくらず接合もしない *Torula* 属の酵母では，その倍数性を定めがたいこととなる。*Torula* 属のものは細胞の形状などから半数性 *Saccharomyces* 属のものがその接合能力を失なって生じたものと考えられていたが，栄養関係の性質からはむしろ二倍体と考えられるふしもあった。Multitarget 説によれば，片対数グラフ紙に画いた生存曲線（Survival curve）の対数減衰部分の外挿から，その倍数性を推定しうるので，単色紫外線（2618 Å）を用い，この方法を *Torula rubra* Saito の倍数性の検査に試みたところ，半数性と推定されるような結果をえた。なお比較に用いた半数性酵母では，照射後それがおかれる培地の組成により生存曲線はちがってくることが明らかとなった。

A New Variety of *Pullularia fermentans* Wynne et Gott

by Minoru YONEYAMA*

米山穣*: *Pullularia fermentans* Wynne et Gott の新変種

Received January 6, 1959

An interesting fungus degrading rutin in a peculiar way[1] was encountered and isolated by Dr. S. Hattori and his co-worker in their laboratory of Tokyo and was sent to the author for identification. At first the newly isolated fungus appeared to belong to the group of "Yeast" because of 1) the abundance of free cell formation of the fungus[2] and 2) its lack of dark pigmentation. Further investigation, however, seemed to justify its inclusion in the genus *Pullularia*, after comparing this fungus with some strains of this genus[3],[4],[5].**

Although dark pigmentation is known up to now as one of the characteristics of *Pullularia*, the new isolate does not produce dark pigment on most media. Also, the appearance of the giant colony of this isolate is peculiar, compared with those of other species of *Pullularia*.

Comparative studies of pigmentation and growth characteristics of all the recognized species of *Pullularia*, including the new isolate, were therefore made under various conditions.

Wynne in 1956[6] proposed a revision of the genus *Pullulria* based almost entirely on biochemical and physiological considerations rather than on morphology. An attempt was therefore made by the author to classify the new isolate according to this new scheme.

Among the species of *Pullularia* studied, the new isolate resembles the genus *Candida*[7] most closely. It may thus be given attention by some mycologists because it might fill a phylogenetic gap between the group of "Yeasts" and some black "Fungi Imperfecti".

Materials and Methods

Materials: 1) the fungus in question isolated from the rutin solution, perhaps air-borne, the Univ. of Tokyo, Japan, 2) a culture of so-labelled *Pullularia pullulans*

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** According to Mr. I. Noguchi, Dr. S. Hattori's co-worker, the property of degrading rutin as seen in the new variety is also observed in the Nagao strain of *Pullularia* and *Pullularia fermentans* var. *fermentans* but not in *Saccharomyces cerevisiae* and very poorly in *Aspergillus niger*. The property of degrading rutin may therefore be a distinguishing characteristic of the genus *Pullularia*.

from the Nagao Institute, Japan,* 3) a culture of *Pullularia werneckii* from Prof. Dr. K. Minoura, Osaka Univ., Japan and 4) a culture of *Pullularia fermentans* var. *fermentans* from Dr. Wynne, Texas Univ., Texas (U. S. A.).

Culture media used: malt extract, malt extract agar, Sabouraud dextrose agar (Difco), dextrose-peptone agar,** corn-meal agar (Difco), nutrient agar (Difco), Littman oxgall agar (Difco), microassay culture agar (Difco), Czapek dextrose agar, ammonium sulfate medium,*** potassium nitrate medium.****

Cultures were made on malt extract, nutrient broth (Difco), in test tubes, Sabouraud dextrose agar (Difco), dextrose-peptone agar, corn-meal agar (Difco), nutrient agar (Difco), Littman oxgall agar (Difco), microassay culture agar (Difco), Czapek dextrose agar, ammonium sulfate medium and potassium medium on both slant and plate, respectively. These cultures were incubated aerobically at 25° and observed on alternate days for the presence of pigmentation and growth. Final readings were made at the end of two weeks. Maximum temperature for the growth of fungus was tested on malt extract and malt extract agar at 37°, after two weeks. Method for testing the production of acid from carbohydrates was that of Wynne, except that a Horiba pH meter was used instead of a Beckman Model G pH meter.

Experimental Results

1. *Classification of the new isolate in the genus Pullularia*:

Classification was based both on morphological and on physiological considerations and comparison with known strains of the genus *Pullularia* (Fig. 1). Blastospores $4\text{-}7\times8\text{-}13\mu$, chlamydospores $7\times10\text{-}13\mu$, arthrospores $6\times13\text{-}15\mu$, cladosporium forms $7\times13\mu$. On Sabouraud dextrose agar, 14 days, at 25°, flat, light colored colony with white surface mycelium, finely fuzzy margin. On malt extract agar, at 25°, at first light pinkish-lilac colored,[8] later dark pigmentation. No dark pigment produced on almost all media such as nutrient agar, corn-meal agar, Littman oxgall agar, microassay agar, Czapek dextrose agar, ammonium sulfate medium and potassium medium. Light colored rings of pellicle in broths such as nutrient, phenol red base broth containing various sugars. No aerial mycelium observed. No growth at 37°. Ferments dextrose, mannose, sucrose, raffinose and xylose.

2. *Pigmentation*:

Observations on the pigmentation of some strains of *Pullularia* including the new

* The author is indebted to the Nagao Institute for supplying the culture.

** The components of this medium were the same as that of Sabouraud dextrose agar (Difco) except that the peptone was purchased in Japan from the Kyokuto-Seiyaku Co.: Bacto-dextrose—10 g., Peptone (Kyokuto-Seiyaku)— 40 g., Bacto agar—15 g., per 1 liter dist. water.

*** Dextrose—30 g., $(NH_4)_2SO_4$—5 g., KH_2PO_4—1 g., $MgSO_4\cdot7H_2O$—0.5 g., Bacto-agar—15 g., per 1 liter of dist. water.

**** Dextrose—30 g., KNO_3—30 g., KH_2PO_4—1 g., $MgSO_4\cdot7H_20$ —0.5 g., Bacto-agar—15 g., per 1 liter of dist. water.

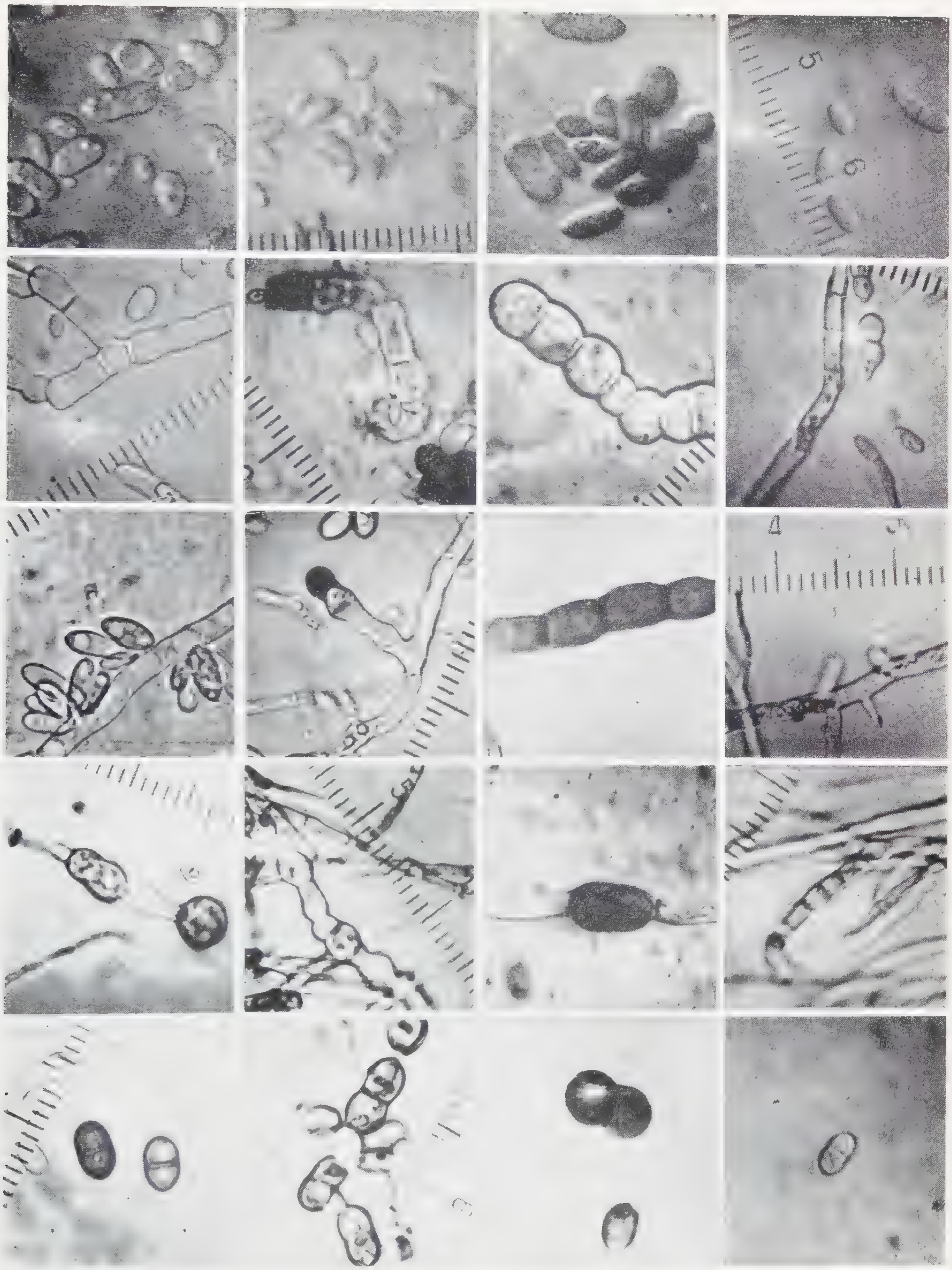

Fig. 1 Microscopic morphology of the *Pullularia*.
Vertical column
1 st row, the new variety.
2 nd row, *P. fermentans* var. *fermentans*.
3 rd row, Nagao. strain, so-labelled *Pullularia pullulans*.
4 th row, *P. werneckii*.
Horizontal column
1 st, blastospores, on Sabouraud dextrose agar, 3 days, at 25°.
2 nd, and 3rd., arthrospores and mycelial hyphae.
4 th, chlamydospores
5 th, cladosporium forms.
All are the same enlargement; one scale unit, 2.5 μ.

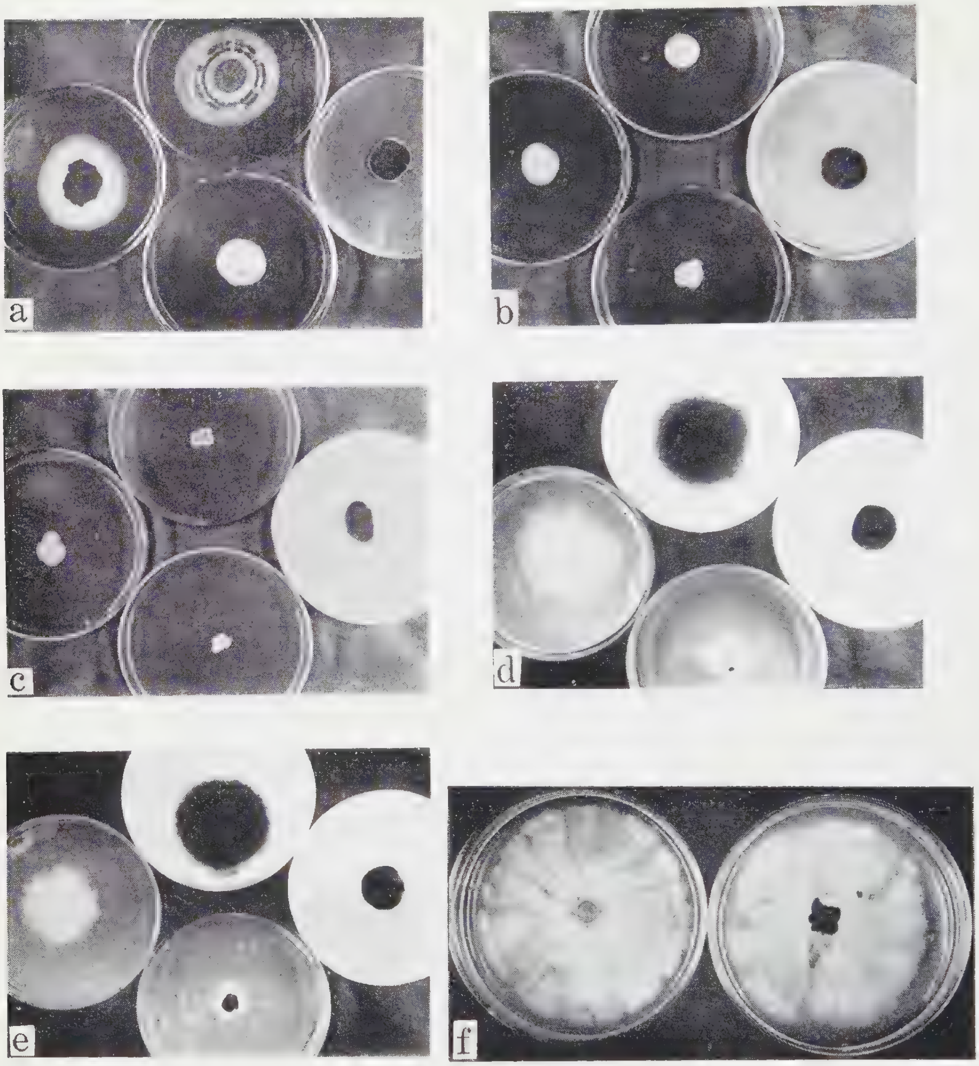

Fig. 2 Giant colonies of some strains of *Pullularia* on various media.
a, b, and c, malt extract agar with NaCl; a, 5%; b, 10%,
c, 12. 5%, respectively.
d, Czapek dextrose agar; e, Potassium nitrate medium;
f, Sabouraud dextrose agar with vitamin B_{12}, 100 μμg. per 1 ml. added.
a e, upper, Nagao strain;
left, new variety;
right, *P. werneckii*;
lower, *P. fermentans* var. *fermentans*.
f, right, *P. fermentans* var. *fermentans*:
left, new variety.

Table 1. Pigmentation of *Pullularia* on various media.*

	Malt extract broth**	Malt extract agar with NaCl added: 0%	5%	10%	12.5%	Sabouraud dextrose agar	Dextrose-peptone agar	Nutrient broth**	Nutrient agar	Corn meal agar	Littman oxgall agar	Microassay culture agar	Czapek dextrose agar	Ammonium sulfate agar	Potassium nitrate agar	Phenol red broth containing various sugars**
P. werneckii	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nagao strain of *Pullularia*	+	+	+	—	—	+	+	+	+	+	+	—	+	+	+	+
P. fermentans var. *fementans*	+	+	—	—	—	+	+	+	—	+	S	—	S	+	S	+
New variety	+	+	+	—	—	—	—	—	—	—	—	—	—	S	—	—

* Readings were made on the slant cultures at the end of two weeks at 25°.
** Pellicle pigmentation.
+ dark pigmentation.
S slightly dark pigmentaion.
— no dark pigmentation.

variety, are shown in Table 1 and partly Fig. 2. It was found that distinctly dark pigmentation occurred on the slant Czapek agar culture of *P. fermentans* var. *fermentans* when some amount of vitamin B_{12} was added (20–100$\mu\mu$g.) (Fig.3).

3. *Giant colony*:

A giant colony of the new variety was different from those of other species of *Pullularia* (Fig. 2-d, e, and Fig. 4). In the case of *P. werneckii*, mycelial phase is so predominant that it is called "mold growth"[6]. On the contrary, the new variety produced more blastospores than mycelial cells. Besides, most of the blastospores did not become mycelial cells which is usual in "mold growth" type strain, but new blastospores sprouted again and again. Besides, it was often seen that a blastospore detatched from the mother cell as if it sprang out (Fig. 5).

4. *The rate of growth in Pullularia*:

The Nagao strain, the new variety, *P. fermentans* var. *fermentans* and *P. werneckii* were in order not only in rapid growth but also in size of giant colonies up to three weeks. This was the case when these fungi were compared with each other grown in parallel on plate cultures of such media as malt extract agar, dextrose-peptone agar, nutrient agar, corn-meal agar, microassay culture agar, Czapek agar, ammonium sulfate medium, potassium nitrate medium (Fig. 2-d, e, and Fig. 4).

* Rubramin, E.R. Squibb L & Donds, New York, Division of Olin Mathieson Chemical.

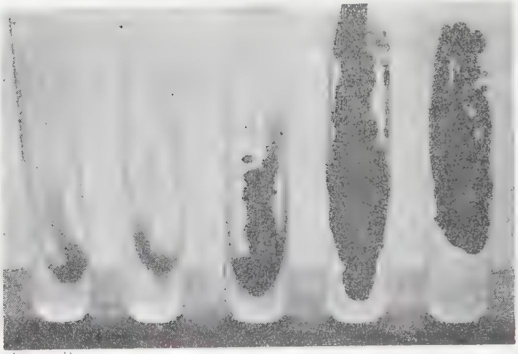

Fig. 3

Fig. 5

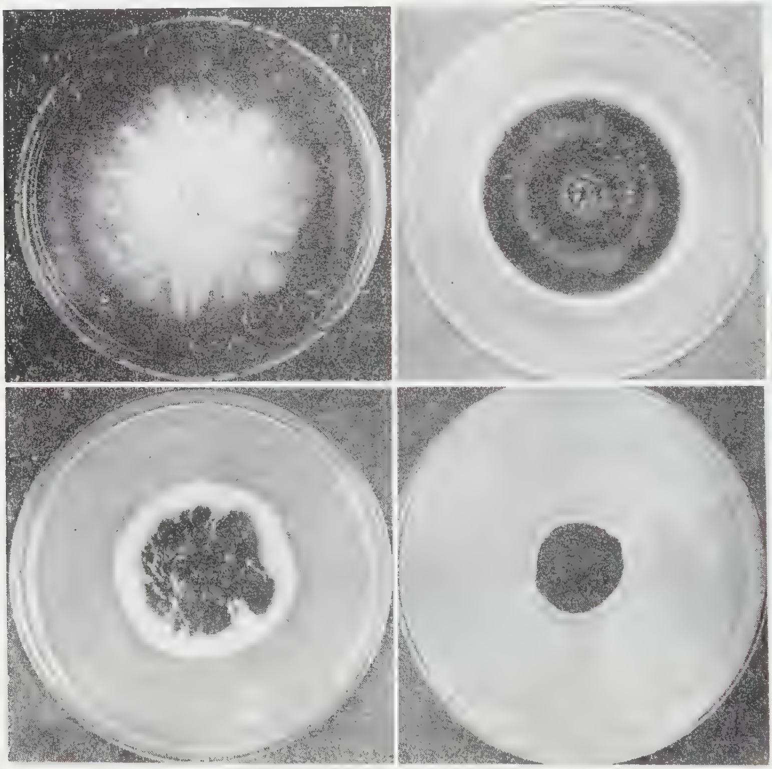

Fig. 4

Fig. 3 Five tube nutritional test of *P. fermentans* var. *fermentans* left to right,
(1)-(3), Czapek agar:
(1), blank,
(2), with vitamin B_{12}, 20 $\mu\mu$g. per 1 ml. added.
(3), with vitamin B_{12}, 100 $\mu\mu$g. per 1 ml. added.
(4)-(5), Sabouraud dextrose agar:
(4), blank,
(5), with vitamin B_{12}, 100 $\mu\mu$g per 1 ml. added.

Fig. 4 Giant colonies of some strains of *Pullularia*, in which emphases were placed on their appearances as well as their pigmentation, on Sabouraud dextrose agar at 25°, two weeks, with exception of one week of Nagao strain. Upper left, new variety; upper right, Nagao strain; lower left, *P. fermentans* var. *fermentans*; lower right, *P. werneckii*.

Fig. 5 Showing the blastospores sprouting from a short mycelial cell of the new variety of *Pullularia*.
Hanging drop culture of malt extract, 25°; upper, original; middle, after 30 minutes; lower, after 40 minutes.

When 100 $\mu\mu$g. per ml. of vitamin B_{12} was added to the Sabouraud dextrose agar plate cultures of the new variety and *P. fementans* var. *fermentans* the growth of the latter fungus was promoted and the appearances of the two fungi bore a resemblance to each other, with the exception of the pigmentation at the central portion (Fig. 2-f).

5. *Salt tolerance*:

As seen in Fig. 2, on malt extract agar added with 12.5% NaCl the growth of *P. fermentans* var. *fermentans* and the Nagao strain were extremely inhibited, but the new variety as well as *P. werneckii* was not affected so much.

Discussion

1. *Classification of the new isolate*:

The new isolate seems to be most closely related to *Pullularia fermentans* var. *fermentans* though the characters of light hyphae and the peculiarity of the giant colony of this fungus would seem to eliminate other varieties of *P. fermentans* described up to now as the variety for this new isolate.

The name of *Pullularia fermentans* var. *candida* is proposed, since it resembles the fungi in the genus *Candida* as stated often in this paper.

Pullularia fermentans Wynne et Gott. var. **candida** Yoneyama var. nov.

Blastosporae $4-7\times 8-13\mu$, chlamydosporae $7\times 10-13\mu$, arthrosporae $6\times 13-15\mu$, cladosporium format $7-13\mu$. Coloniae in agarico dextroso-peptone planae, pallide roseo-lilacinae, mucosae, area centrali elevata irregulariter tuberculata, margine subtiliter fimbriatae. Pigmentum produtum and centrum in agarico maltoso et dextroso Sabouraudii: Nullum pigmentum in agaro farinae frumanti, in agaro Littmani, in agaro nutriente, inagarum Czapekii. Non crescit cum 37°. Glucosum, mannosum, maltosum, sucrosum, raffinosum et xylosum fermentat.

Isolata in culturis ex solutione rutinorm in Tokiensi, Japonia.

2. *Pigmentation*:

The following schema would help to explain the occurrence of pigment in species belonging to *Pullularia*:

$$\begin{array}{ccccc} O & \longrightarrow & P & \longrightarrow & Q \\ & \uparrow & & \uparrow & \\ & X & & Y & \end{array}$$

O represents a genic factor which would cause the pigmentation in all species of *Pullularia*, leading to Q which represents pigmentation. P is something which would play a role between O and Q. X is a substance which may be responsible for processing of the course O→P, and Y, another substance for the course P→Q. It seems to be a characteristic of the pigmentation in *Pullularia* that the course O→P→Q is followed.

Dark coloring strains such as *P. pullulans*, *P. werneckii*, and the Nagao strain seem to be capable of completing this course, that is, they have strong genic factors and

are able to produce both X and Y on most media; whereas the new variety seems not to complete this course, that is to say, it has a certain genic defect or failure as regards the course, so that it does not produce X, Y, or both of them[9),10),11),12)]. That the pigmentation of the new variety does occur on malt media is not easy to explain, but X or Y, or a substance or substances which might induce occurrence of X or Y, in the fungus may be present in malt extract. *P. fermentans* var. *fermentans* seems to have an intermediate position between the two types.

3. *Giant colony*:

A giant colony is, as Bornside[13)] pointed out, regarded as a group of cells arranged as a result of fungal development and environmental conditions. So the difference between two giant colonies of the two species of fungi on the same solid media may possibly due to two factors: 1) the way, in which the fungus tends to produce either yeast-like cells or mycelial cells, 2) mode of vegetative reproduction, i. e budding or mycelial elongation plus branching.

Regarding the conversion of the mycelial cells to yeast-like cells, there appeared several reports[14),15),16),17),18)], which ascribed the conversion to the effect of cultural conditions. However, yeast-like cells which appear predominantly and the peculiar mode of their sprouting as seen in the culture of the new isolate may be used as taxonomic criteria[19)], when compared with other species of *Pullularia* under a certain condition.

4. *The growth of the species in Pullularia*:

The fact that the growth of *P. fermentans* var. *fermentans* could be promoted on Sabouraud dextrose agar by adding a little quantity of vitamin B_{12} suggests that a simple chemical substance may be of some relation to the growth of the fungus as is the case with the production of dark color. It is therefore apparent that there exists an intimate affinity between *P. fermentans* var. *fermentans* and the new isolate.

5. *Salt tolerance* (*Tolerance in hypertonic medium*):

This property should be sufficient in making a distinction between the new variety and *P. fermentans* var. *fermentans* or the Nagao strain; it is likely to assume its natural habitat is different from those of other species. However, it is regrettable to say that the natural habitat is unknown to us up to now.

The following schema may be finally drawn:

Moving from the right (dark fungi) to the left (the yeast group), there is decrease in pigmentation and rate of the mycelial phase. Fermentation ability is present in *P. fermentans* and the new variety and P-F (center of the schema, which is not an actual species but an imaginative one). Many examples which corresponded to P-F were discovered by Wynne and the Nagao strain seemed to be an example of this type.

Summary

1. A taxonomic study was made of an organism isolated from rutin solution and to which the name of *Pullularia fermentans* var. *candida* was given by the lack of dark pigment of hyphae on most media and by the peculiar appearance of a giant colony from abundance of blastospores and the mode of their sprouting.

2. Comparison of dark pigmentation and giant colonies of the species of *Pullularia* was made, respectively; the lack of dark pigmentation and the peculiarity of a giant colony of the new variety were considered to be worthy of using as taxonomic criteria.

3. The position of the new variety which seems to be an intermediate form between the genus *Pullularia* and the genus *Candida* (Yeast) was discussed.

4. A substance which might be responsible for possible pigmentation or growth of the *Pullularia* was presumed.

Acknowledgments

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摘　　要

東大の服部教授の研究室で，ルチンを分解する菌が分離されたので，その同定を行なった。本菌は酵母に非常によく似ているようだったが，培養条件により *Pullularia* の特徴の片鱗を示したので，長尾研究所からの *Pullularia pullulans* と比較してみて，*Pullularia* に属させるべきものとわかった。

Pullularia は従来 *P. pullulans* と *P. werneckii* の 2 種だけ知られていたが, Wynne (1956) は ホジキン氏病患者の病巣から *Pullularia* を分離し，それがある炭水化物から酸を生成するので *Pullularia fermentans* という新種をもうけて *Pullularia* 属の定義を修正するよう提唱した。その修正の基礎は *Pullularia* の分類のを規準として炭水化物からの酸形成能を採用することにある。東京からの本菌は，その形態と Wynne の提唱による分類規準とからみて *P. fermentans* var. *fermentans* にもっとも近縁であった。しかしたいていの培地上で *Pulluaria* の特徴である暗色色素を形成しないこと，巨大コロニーの様相が特異であることから新変種とし，*Pullularia fermentans* var. *candida* とした。なお本菌は高張培地にもかなりよく耐えるが，それは比較に用いた *P- fermentans* var. *fermentans* や長尾研究所からの菌 so-labelled *P. pullulans* とも異なるので，この新変種の天然のすみかは，従来しられている *Pullularia* のすみかとは異なったものと思われる。

この研究では *Pullularia* の四つの菌株について，暗色色素の形成とコロニーの様相が種々の条下件で比較されたが，*Pullularia* の暗色色素形成と生長の速度とが，ある場合には，簡単な化学物質によって支配されることがわかった。それで *Pullularia* のある菌株は microbioassay の研究材料としてもよいのではないかと思われる。

The Effects of Phosphorus and Calcium Deficiency on the Growth and Dry Matter Production in *Chrysanthemum coronarium* var. *spatiosum* in Water Culture

Relations of Plant Communities to Edaphic Factors with Special Reference to Mineral Nutrition. II.

by Yasuhiko TEZUKA*

手塚泰彦： [illegible] に及ぼす影響。
無機栄養からみた植物群落と土壌条件の関係 II.

Received December 25, 1958

In a previous paper[1] the author has clarified that the decisive role of nitrate ion on the plant growth was not in its concentration but in its whole available amount, and he explained the depression in growth caused by nitrogen deficiency, on the basis of dry matter production[2]. Now it should be an interesting problem to clarify whether phosphate and calcium ions influence the plant growth in the same way as nitrate ion does.

In the present paper the following subjects are dealt with; the relations of plant growth to the amounts of phosphorus and calcium supplied, the significance of ion concentration for the ion absorption and plant growth, and the changes of photosynthetic activity and dry matter production caused by phosphorus and calcium deficiency.

Material and Method

Chrysanthemum coronarium var. *spatiosum* was used throughout the experiments, because of the fitness of the plant for the nutrient experiment, as mentioned previously[1]. The experiments were divided into two series of cultures, i. e. phosphorus and calcium experiments. In both cultures, the procedure for the establishment of seedlings, culture method, and constitution of standard culture solution used were the same as shown in the previous paper[1].

Five plants per pot were harvested at random every 10th day. After weighing the fresh weight of leaves, stems and roots, the materials were oven-dried at 80° for determination of dry weight of each organ and for chemical analyses for phosphorus, calcium, iron, etc.. Those chemical analyses and the measurements of photosynthetic activity and respiration were done in the same ways as described in the previous paper[1].

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I. Phosphorus experiment

In this experiment, the source of phosphate ion was given as KH_2PO_4. The culture experiments were carried out for 60 days from November 16, 1953 in a frame. Three culture sets were prepared; one of which contained no phosphorus at all (designated as O–P), and another contained sufficient amount of phosphorus (KH_2PO_4 0.05g./l.) throughout the culture duration (designated as +P). The other contained the same amount of phosphorus as the +P set for the first 30 days, but after that the plants were cultivated under the no-phosphorus condition (designated as —P) for following 30 days.

a) The effects of phosphorus deficiency on the growth.

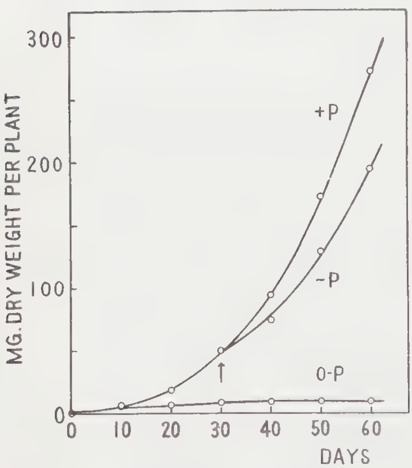

Fig. 1. Growth curves under various phosphorus treatments. See text.

The initial mean dry weight of a seedling was 1 mg.. The plants of the O–P set continued their slow growth until 30th day of cultivation, when they reached about 10 mg. in maximum dry weight. This growth may be due to phosphorus stored in the cotyledons or some contaminations of the used chemicals. On the other hand, the plants of the +P set grew normally with a steady growth rate until the end of the culture. While, the plants of the —P set grew more slowly than the +P set plants after the transfer to the no-phosphorus solution, but no stoppage of growth was observed, probably being due to the phosphorus already accumulated in the tissue in the preceding cultivation. These features are shown in Fig. 1.

In order to determine whether phosphorus has its effects on plant growth in concentration or in whole amount, an additional culture experiment was carried out in the autumn of 1955. Two pots of low phosphorus concentration (initial concentrations were 1.76 and 1.80 p.p.m.) were prepared, together with a control of 50 p.p.m. phosphorus. The procedure was the same as the above experiment. It can be seen from Fig. 2 that phosphorus concentrations in the solution diminished until about 0.3 p.p.m. linearly with dry weight increase of the plants, and there was no difference in the plant dry weight between high and low phosphorus levels, though the phosphorus of the latter was perfectly exhausted by the 37th day, and the absorption rate of phosphorus decreased after the 20th day or below 0.3 p.p.m.. This result agrees on the whole with Olsen's[3),4),5)], who found that phosphate absorption by rye and kitchen kale was independent of concentration above 0.003 m.eq./l. (about 0.1 p.p.m. of phosphorus). It is difficult, however, to elucidate the reason why there was no difference in plant growth between high and low phosphorus

levels after 37th day, in contrast with the results illustrated in Fig. 1.

b) Chemical contents in plant tissues.

Symptom of phosphorus deficiency was visual in dark greening of leaves, especially in older leaves. This fact has been well known[6]. This symptom was due to high chlorophyll content; for example, chlorophyll was 7.8 mg. per 10 g. fresh leaves of the +P plants, while of the −P it was 14.4 mg., on the 60th day of culture. According to Rediske and Biddulph,[7] dark greening of leaves caused by phosphorus deficiency appears to be not the result of direct acceleration of chlorophyll formation by phosphorus deficiency itself but that of the increased iron-availability caused by phosphorus deficiency. Their elucidation seems to hold good for the present experiment, because iron content of 0.40 mg., detected in 1 g. dry weight of the phosphorus-deficient leaves after 60 days cultivation, was exceedingly higher than that of 0.01 mg. in the phosphorus-sufficient leaves.

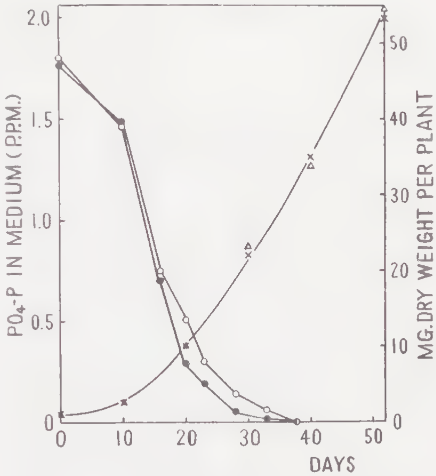

Fig. 2. Plant growth and changes of phosphorus in medium.

—△— : dry weight of control (50 p. p. m.) plant.

—×— : dry weight of experimental plant.

—●— : medium containing 1.76 p. p. m. of phosphorus.

—○— : medium containing 1.80 p. p. m. of phosphorus.

Phosphorus concentrations in leaves, stems and roots were analysed at successive stages of growth (see Table 1). Phosphorus levels in tissues of the O-P plants decreased rapidly during the first 10 days and slowly later on, and those of the −P plants also decreased markedly after transfer to the no-phosphate solution, though the phosphorus levels in each organ of the +P and the −P plants were strikingly higher than those of the O-P plants. The fact that phosphorus concentrations in the plant tissues suffering from phosphorus deficiency decrease up to a certain minimum

Table 1. Contents of total phosphorus at successive stages of growth. (Per cent on dry weight basis)

	Phosphorus treatments	0 day	10 days	20 days	30 days	40 days	50 days	60 days
Leaves	+P	—	0.69	0.51	0.48	0.40	0.40	0.37
	−P	—	—	—	0.48	0.31	0.14	0.13
	O-P	—	0.14	0.13	0.10	0.08	0.05	0.05
Stems	+P	0.44	0.39	0.42	0.40	0.38	0.34	0.29
	−P	—	—	—	0.40	0.36	0.22	0.27
	O-P	0.44	0.13	0.10	0.13	0.10	0.06	0.06
Roots	+P	0.38	0.85	1.01	0.78	0.92	0.91	0.55
	−P	—	—	—	0.78	0.61	0.47	0.35
	O-P	0.38	0.15	0.17	0.09	0.07	0.09	0.09

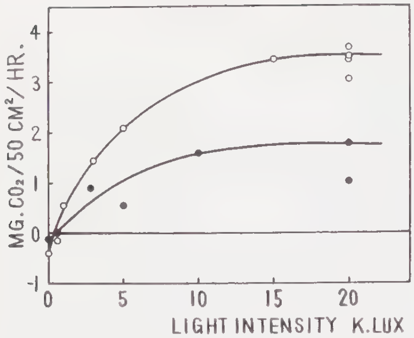

Fig. 3. Light-photosynthetic curves of +P and -P leaves after 60 days cultivation. Temperature 20°.

—○— : +P, —●— : −P.

level, may be useful for the identification of phosphorus deficiency in field-grown plants.

c) Photosynthesis, respiration and dry matter production.

Light-assimilation curves of the +P and the −P plants after 30 days from the transfer to the no-phosphorus solution are shown in Fig. 3. Phosphorus deficiency caused a marked decrease in the photosynthetic activity, in spite of the high chlorophyll content of the leaves. This result does not agree with a well-known parallelism between chlorophyll content and photosynthetic activity[8),9)]. A marked decrease in respiration caused by phosphorus deficiency can be seen from Table 2. The decrease in leaves was the greatest of various organs.

Table 2. Respiratory activities of each organs of the +P and the −P plants after 60 days. (at 20°)

	Leaves (mg. CO_2/50cm²./hr.)	Leaves (mg. CO_2/100mg. D.W/hr.)	Stems (mg. CO_2/100 mg. D.W./hr.)	Roots (mg. CO_2/100 mg. D.W./hr.)
+P	0.38	0.41	0.33	0.32
−P	0.12	0.10	0.23	0.26

Furthermore, gross production and net production per day were calculated from the photosynthetic curves and a diurnal curve of light intensity in December in Tokyo, on the 60th day of cultivation (cf. previous paper as to calculation method). Major values are indicated in Table 3. The relative daily net production of the +P plants was 6.6% of their dry weight and that of the −P plants 4.0%. These values correspond fairly well to the gradients (7% and 5%, respectively) of growth curves in Fig. 1. Moreover, the value of 6.6% of the +P plants is rather low, which seems to explain the empirical fact that this crop grows in the field more slowly than such crops as rape and spinach do.

Table 3. Daily production of +P and −P plants after 60 days. (at 20°)

	Dry Weight (mg.) Leaf	Stem	Root	Total	Leaf area (cm².)	Pg (mg.)	R (mg.)	Pn (mg.)	%Pn
+P	179	39	55	273	73.2	22.8	4.7	18.1	6.6
−P	126	30	37	193	51.7	10.4	2.7	7.7	4.0

II. Calcium experiment

Four calcium levels of 0, 2, 5 and 15 p.p.m. (added as $CaCl_2$) were prepared. The culture experiment was carried out for 50 days, from April 29, 1955, without renewing the culture solutions.

a) Amount of calcium and plant growth.

In this experiment the initial mean dry weight of a seedling was 2.2 mg.. The growth curves in Fig. 4. obtained under various calcium supply show that the maximum plant weights are roughly proportional to the total amount of calcium supplied in the culture solution. It is noticeable that cations, such as calcium, influence the plant growth in the same way as anions such as nitrate and phosphate do, and it might be expected that the growth of the plant is not determined by the concentration itself but by the whole amount of cations. These features are very important for the quantitative approach in field ecology.

b) Calcium deficiency and calcium content in the plant tissue.

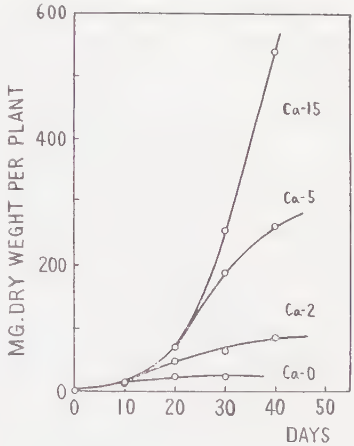

Fig. 4. Growth curves under various calcium supply. See text.

Table 4. Ca'cium contents (per cent on dry weight basis) and the ratio of non photosynthetic (stem+root) to photosynthetic system (leaf) (dry weight basis) after 30 days.

		Ca-O	Ca-2	Ca-5	Ca-15
Ca-	Leaves	0.37	0.37	0.39	0.63
	Stems	0.54	0.36	0.32	0.45
content	Roots	0.69	0.49	0.42	0.41
Stem+Root/Leaf		1.33	0.82	1.07	1.07

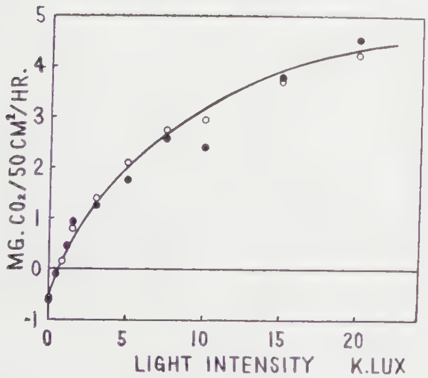

Fig. 5. Light-photosynthetic curves of Ca-5 and Ca-15 leaves after 30 days cultivation. Temperature 20 .

—●— : Ca 5, —○— : Ca-15.

The plants deficient in calcium showed the characteristic symptoms of calcium deficiency i. e. necrosis of the apical buds and abnormal development of each leaf, suggesting immobility of calcium within plant organs. This is interesting along with the fact that no great difference was found among :calcium contents of the plant tissues of various calcium levels as well as of various organs (see Table 4).

c) Photosynthesis and calcium deficiency.

Photosynthetic curves per unit leaf area obtained in the leaves of the Ca–5 and Ca–15 plants on the 30th day of cultivation show that there no was conspicuous difference between them, although the marked difference was observed in the plant dry weights (see Fig. 5). However, on the dry weight basis, photosynthetic avtivity of

the calcium-deficient plants decreased considerably on account of their succulent leaves (for example, dry weight of 100 cm.[2] leaves of the Ca–5 and that of the Ca–15 plants after 40 days cultivation was 426 and 293 mg., respectively.) The decrease in growth caused by calcium deficiency seems mainly to be attributed to the retardation in development of leaf area, although further experimentations should be required to elucidate perfectly the growth depression caused by calcium deficiency, based on the dry matter production.

Summary

Regarding the relations of the growth of *Chrysanthemum coronarium* var. *spatiosum* to the amount of phosphorus and to that of calcium under water culture conditions, the following results were obtained:

1. The effects of phosphate and calcium ions on the plant growth were considered not to be in its concentration but in its whole amount which is available for the plant.

2. Phosphorus deficiency causes the increase in chlorophyll content in leaves, consequently dark greening of leaves. On the other hand, the symptom of calcium deficiency is the necrosis of apical buds and retardation in development of each leaf.

3. Phosphorus deficiency results the remarkable reduction of photosynthetic activity per unit leaf area and the decrease in respiratory activity of each organ. Calcium deficiency seems to give no such effect on photosynthesis per unit leaf area, but brings about a considerable decrease of photosynthesis per unit dry weight.

4. Decrease in plant growth caused by phosphorus deficiency was explained in the light of dry matter production.

Grateful acknowledgment is made to Prof. K. Hogetsu for his helpful advice and discussion and to Prof. M. Monsi for his many valuable suggestions. A part of the present investigation was financed by a grant-in-aid from the Resaerch Fund of the Ministry of Education.

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摘　　要

シュンギクを水耕し，その生長（乾燥重量の増加）と物質生産に及ぼす燐およびカルシウム欠乏の影響を調べた。

燐の実験では，培養のはじめから燐をまつたく与えないもの（O－P），培養をはじめてから30日間燐をじじゅうぶんに与え，その後燐をまつたく含まない培養液に移したもの（－P），培養期間中（60日間）じゅうぶんに燐を与えたもの（＋P）についてその生長を測定した。また附加的な実験で培養液中の燐の濃度変化と生長の関係を調べた。

またカルシウムの実験では，0, 2, 5, 15 p.p.m. の濃度（培養液は更新しない）で培養し生長を追跡した。

燐とカルシウムの両方の実験で，培養後の一定の時期に光合成能力，呼吸等を測定し，生産量を理論的に計算した

これらの実験の結果をまとめると：

1. 最大生長量は，硝酸イオンの場合と同様に，燐酸イオンやカルシウムイオンの濃度には左右されず，絶対量によって決定されるものと考えられる

2. 燐の欠乏は葉の葉緑素含量の増加，すなわち暗緑色化をひき起こすが，これに対し，カルシウム欠乏の徴候は特徴的で，頂芽の枯死となってあらわれる

3. 燐の欠乏は光合成と呼吸のいちじるしい低下をひき起こす。カルシウムの欠乏は単位面積あたりの光合成能力には影響を与えないようであるが，単位重量あたりの光合成能力を低下させる。

4. 燐の欠乏によっておこる生長の低下は光合成，呼吸の変化による物質生産の変化をもとにして説明された。

花粉の発芽，花粉管の伸長および原形質流動におよぼすアルコールの影響

高　見　　亘*

Wataru Takami: Effects of Alcohols on the Germination of the Pollen Grain, the Growth of the Pollen Tube and the Plasma Streaming

1958 年 10 月 8 日受付

花粉の発芽の促進剤については多くの研究があり，筆者[1] も多くの無機塩を，しょ糖寒天培地に適当な濃度で加えると花粉管の伸長が促進されることを報告した。最近 Moyer[2] によって，メタノール，エタノール，イソプロピルアルコールが糸状菌による有機酸，ことにクエン酸の生成をいちじるしく促進することが報告され，問題にされている。しかし，アルコールの花粉の発芽におよぼす影響についてはまだ知られていないので，一価のアルコールのうち，どれが促進作用を呈するか，またその程度などについて調べた結果を報告する。

実験の方法と結果

発芽の方法は培地にアルコールを加えるために，Van Tieghem の湿室をもちい，したがって花粉は水またはしょ糖液で発芽するものを選んだ。実験したアルコールは (1) メチル，(2) エチル，(3) *n*-ブチル，(4) *sec*-ブチル，(5) *t*-ブチル，(6) イソブチル (7) アミル，(8) イソアミル，(9) *n*-プロピル，(10) イソプロピルアルコールと (11) シクロヘクサノールである。ムラサキツユクサによる予備実験の結果，0.05%のメタノールとエタノールに促進作用が認められたので，control として水 (pH. 6.0)。または 10% か 15% のしょ糖液を用い，添加アルコールの濃度は 0.005, 0.01, 0.05, 0.1% とした。原則として同じ花の花粉をまいて室温で発芽させ，一定時間後に 20 個前後の花粉管の長さを ×150 でミクロメーターによって測り (12 目盛が 130 μ)，各平均値を control に対するパーセントで表わした。また発芽率は 100 個以上の花粉について求めた。

(1) 花粉管の伸長におよぼす影響： 観察した花粉と培養時間はホウセンカ 20 分，ムラサキツユクサ 30 分，ツバキ，ジャノヒゲ，チヤ，ヤマブキ. 各 60 分，チューリップ，ボケ各 90 分で，花粉管の伸長の割合は第 1 表に示すようであった。ムラサキツユクサは変動が多く，しょ糖の 2, 3, 5, 7, 10% 液で開花直後 (午前 3 時) の花粉について，最適濃度を調べても確定的ではないが，10% が最良のようで，表には一例を示した。また，ホウセンカも発芽の速さに変動が多く，場合によっては 30 分後に観察した方がよく，やくのもっとも外側の花粉は異常に花粉管の伸長が速いこともあり，そのようなものは統計から除いた。

上の結果からエタノールとメタノールに促進作用が認められたので，さらにチャ (60 分)，ビョウヤナギ (90 分)，ホウセンカ (30 分) について，促進作用がどの程度に変動するかを調べると，第 2 表のようであった。

この促進作用はしょ糖寒天板 (しょ糖 10%，寒天 1.5%，pH 6.0) でも現われ，ムラサキツユクサの場合には，メタノールの添加量を 0.01, 0.05, 0.1% にすると 30 分間培養後の花粉管の伸長はそれぞれ 1.21, 1.28, 1.10 倍となり，また，チューリップを 15% しょ糖液とそれにメタノールを 0.05% になるように加えたもので 24

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Table 1. Pollen tube growth in alcohol of various concentration expressed as percentage of growth in control.

Flower	Control	Alcohol Conc.	Ethanol	Methanol	*n* Butyl	*s*-Butyl	*t* Butyl	*iso* Butyl	Amyl	*iso*-Amyl	*n*-Propyl	*iso*-Propyl	Cyclo-hexanol
Tradescantia reflexa	Sucrose 10% 26°	0.01%	126	126	136	151	burst	116	burst	burst	83	194	78
		0.05	245	135	69	181	〃	141	〃	〃	108	129	116
		0.1	130	154	118	118	〃	91	〃	〃	106	99	144
Impatiens balsamina	Water 24°	0.01	150	133	140	153	250	134	330	120	103	139	117
		0.05	230	493	123	127	180	134	253	210	70	139	161
		0.1	290	113	120	170	160	89	140	127	123	89	207
Camellia japonica	Water 21°	0.01	126	129	278	123	134	163	97	152	189	170	211
		0.05	196	270	148	119	123	145	70	44	189	211	89
		0.1	130	108	74	130	111	167	52	44	93	130	96
Tulipa Gesneriana	Sucrose 15% 20°	0.01	100	122	68	149	154	87	74	90	108	105	65
		0.05	156	155	136	140	129	88	120	73	95	131	70
		0.1	115	139	103	123	126	119	96	82	110	burst	60
Ophiopogon japonica	Water 20°	0.01	106	85	109	107	164	154	96	87	117	44	117
		0.05	117	117	96	117	124	139	93	106	91	48	115
		0.1	109	74	104	122	100	124	94	74	93	74	109
Thea sinensis	Water 25°	0.01	114	128	115	114	108	93	110	109	73	49	104
		0.05	128	108	115	98	83	95	107	80	90	74	80
		0.1	85	64	108	108	69	54	107	36	88	47	98
Kerria japonica	Water 20°	0.01	90	103	94	100	83	79	66	89	73	104	91
		0·05	77	77	100	71	78	73	68	59	78	87	78
		0.1	61	58	90	79	65	68	71	59	56	74	69
Chaenomeles lagenaria	Water 20°	0.01	75	68	103	71	113	70	74	41	103	82	87
		0.05	87	91	49	71	120	100	74	97	60	68	100
		0.1	68	72	56	84	98	104	59	59	51	97	116

Table 2. Pollen tube growth in ethanol and methanol expressed as percentage of growth in control.

Flower	Control	Alcohol Conc.	Ethanol	Methanol
Thea sinensis	Water 22°	0.01% 0.05 0.1	108 316 217	158 158 408
	〃	0.01 0.05 0.1	106 69 57	89 120 123
	〃	0.01 0.05 0.1	110 100 170	200 370 150
	〃	0.01 0.05 0.1	213 200 288	175 235 175
	〃	0.01 0.05 0.1	104 158 64	127 98 107
Hypericum chinense	Water 20°	0.01 0.05 0.1	190 235 145	205 225 140
	Water 25°	0.01 0.05 0.1	72 116 56	64 114 146

Flower	Control	Alcohol Conc.	Ethanol	Methanol
Impatiens balsamina (small)	Water 25°	0.01% 0.05 0.1	150 204 115	96 117 269
	Water 26°	0.01 0.05 0.1	274 215 308	192 212 131
	Water 26°	0.01 0.05 0.1	309 368 burst	182 262 112
	Water 27°	0.01 0.05 0.1	303 188 burst	87 190 64
Impatiens balsamina (big)	Water 24°	0.01 0.05 0.1	210 240 270	160 210 540
	Water 24°	0.01 0.05 0.1	314 258 148	175 162 205
	Water 25°	0.01 0.05 0.1	212 648 149	203 164 403

Table 3. Percentage of pollen germination in alcohols of various concentration

Flower	Control	Alcohol Conc.	Ethanol	Methanol	*n*-Butyl	*s*-Butyl	*t*-Butyl
Hypericum chinense	3 (Water 20°)	0.01 0.05 0.1	23 32 25	23 45 32			
Camellia japonica	76 (Sucrose 15% 18°)	0.005 0.01 0.05 0.1	79 63 71 54	86 75 73 72	82 66 75 69	85 86 89 52	83 80 87 68
	11 (Water 21°)	0.01 0.05 0.1	17 37 8	20 57 9	35 39 10	19 18 25	38 33 29

Flower	Control	*iso*-Butyl	Amyl	*iso*-Amyl	*n*-Propyl	*iso*-Propyl	Cyclohexanol
Camellia japonica	11 (Water 21°)	42 12 28	5 9 6	20 0 0	23 25 9	30 35 29	42 6 34

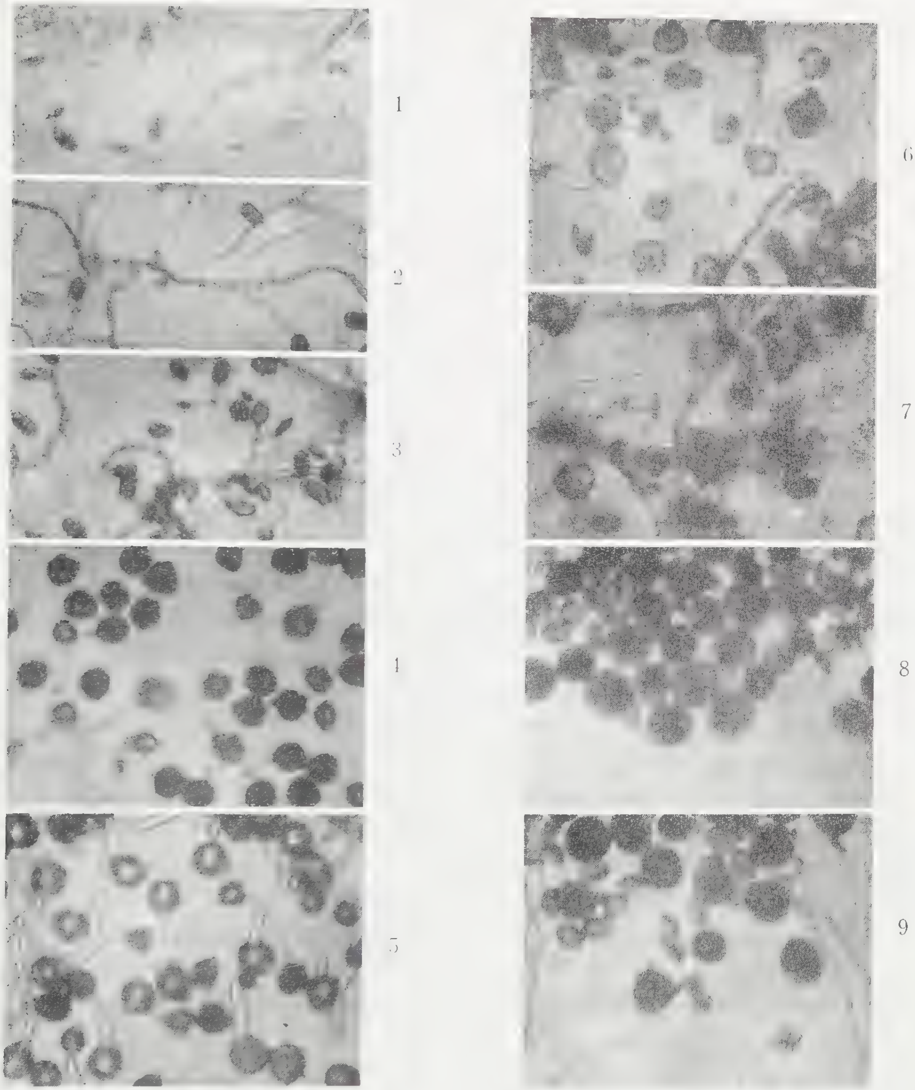

Fig. 1. Promoting effect of methanol and ethanol on pollen germination by Van Tieghem's apparatus. 1-3 *Tradescantia reflexa* after 2 hrs. 1) in 10% sucrose solution, 2) in 10% sucrose solution containing 0.01% ethanol, 3) in 10% sucrose solution containing 0.01% methanol. 4-5 *Camellia japonica* after 3 hrs. 4) in water, 5) in 0.05% methanol solution. 6-7 *Tuliba Gesneriana* after 24 hrs. 6) in 15% sucrose solution, 7) in 15% sucrose solution containing 0.05% methanol. 8-9 *Tritonia crocosmaeflora* after 24 hrs. 8) in 15% sucrose solution, 9) in 15% sucrose solution containing 0.05% methanol ×83

Table 4. Effects of alcohols on the plasma streaming in the hair of the stamen of *Tradescantia reflexa* Rafin. at 25° (+means stopping)

Conc.	Ethanol	Methanol	*n*-Butyl	*s*-Butyl	*t*-Butyl	*iso*-Butyl	*n*-Propyl	*iso*-Propyl
1%	−	−	−	−	−	−	−	−
2	−	−	+	−	−	−	−	−
3	−	+	+	+	−	+	−	−
4	−	+	+	+	+	+	−	−
5	−	+	+	+	+	+	+	+

時間培養すると，メタノールを添加したものでは control にくらべて 2.7 倍になった。

(2) 発芽率に対する影響： ムラサキツユクサの花粉を 10% しょ糖液，室温 23° で発芽させたとき 30 分後の発芽率は 8% で小さいが，エタノールとメタノールを 0.1% になるように加えると，発芽率はそれぞれ 12%，16% 程度に増大した。ビョウヤナギ (90 分後) とツバキ (60 分後) の場合に測定した結果は第 3 表のようであった。ツバキの花粉をしょ糖液で発芽させた場合には，アルコールを添加しても発芽率はせいぜい 10% 位しか増大しないが，水で発芽させた場合には，エタノール，メタノール，*n*-ブチル，*t*-ブチル，などを添加すると 20% 以上増大した。ビョーヤナギは水では発芽率がはなはだ小さいが，エタノール，メタノールを添加すると発芽率は 20% 以上増大した。

発芽率が良好の場合には，花粉管の伸長も大きいのは当然で，エタノールとメタノールを加えた培地では Fig. 1 に示すように control に比べてよい。ムラサキツユクサの発芽率は 10% しょ糖液でははなはだ小さく 4% 程度であるが，エタノールまたはメタノールを 0.01%になるように加えると発芽率は 20% 程度になり，花粉管の伸長も増大した。(第1図1〜3)。特にアルコール添加の影響がいちじるしく見られたのはツバキの花粉で，水では花粉管の伸長ははなはだ少ないが，メタノールを 0.05% になるように加えると花粉管の伸長が増大した。(第1図 4〜5) チューリップではツバキほどいちじるしくはないが，15% しょ糖液に，メタノールを 0.05% になるように加えると 24 時間後には花粉管の伸長が増大された，(第1図 6〜7) またヒメヒアオギズイセンの花粉は 15% しょ糖液でも全部破裂してしまうが (第1図 8)，メタノールを 0.05% になるように加えると同 9 図に示すように破裂しないで，発芽率ははなはだ小さいが発芽することが観察された。

(3) 原形質流動に対する影響： ムラサキツユクサの花粉の発芽したものを水を培地にしてスライドにとり，原形質流動の速さが 28° で 2.8 μ/sec. と一応一定したものを，0.05% のメタノールでおきかえると速さは 7.1 μ/sec. となった。また，ムラサキツユクサのおしべの毛では 28。のとき水中で 4.2 μ/sec. のものを，0.05% のメタノールでおきかえてもすぐには変らないが，長く放置して 25° のとき水中で 0.7 μ/sec. となったものを 0.05% のメタノールでおきかえると 2.6 μ/sec. と急増した。さらに，ムラサキツユクサのおしべの毛の先端細胞を用いて種々なアルコール中での原形質流動がアルコールのどの程度の濃さで止まるかを調べた結果を第 4 表に示す。

結論および論議

上の実験の結果によれば，多くのアルコールは花粉の発芽を促進させる性質をもっていることがわかる。その最適濃度は培地によって多少異なるが0.01〜0.05% 程度で，培地がしょ糖液としょ糖寒天板とでは最適濃度の差はないようである。しかし，水としょ糖液とでは最適濃度はちがうようで，例えばチャの花粉では，15% のしょ糖液を control として，メタノールを 0.01, 0.05, 0.1% となるように加えると花粉管の伸長比(%)は 107, 211, 200 となって最適濃度は 0.05〜0.1% である control を水とした場合には，第1表からわかるがように最適濃度は0.05〜0.01% である，また，ホウセンカでは大きい植物の花粉は小さい植物より

も最適濃度が大きいことが第2表から知られる。

ヤマブキとボケはアルコールの促進作用がほとんど見られなかったが，そのほかのものではエタノールとメタノールがいちばん安定した促進作用をもっている。ブタノール4種も促進作用をもつといえるが，アミルアルコール，プロピルアルコールはホウセンカのような例外的のものを除けば害作用があり，シクロヘクサノールでは確定していない。

糸状菌によるクエン酸生成の場合にアルコールを 3% 程度培地に加えると，最初の菌体の発育は害されるが，クエン酸の生成量はいちじるしく増し，それは酵素系を刺戟するためであるとされているが，くわしい理由はまだ知られていない。花粉の場合には，酵素系を刺戟するとともに吸水を調整するためと考えられる。これはムラサキツユクサのように破裂しやすい花粉では，エタノールやメタノールなどを加えると破裂が少なくなることや，ヒメヒアオギズイセンのようにしょ糖液だけでは破裂してしまうものでも，メタノールを加えると発芽することによって知られる。このように，しょ糖液だけでは破裂するがアルコールを加えると発芽するような例は，これまでのところその他にはえられなかった。この実験終了後にも，人工培地における花粉の発芽については本誌にも報告されており[3],[4]，興味がある問題であろう。

Summary

The effects of alcohols on the pollen tube growth, the germination percentage and the plasma streaming in the hair of the stamen of *Tradescantia reflexa* Rafin. were observed. Alcohols tested were as follows: Methyl, ethyl, *n*-butyl, *sec*-butyl, *tert*-butyl, *iso*-butyl, amyl, *iso*-amyl, *n*-propyl, *iso*-propyl alcohols and cyclohexanol. Various promoting and inhibitory effects were obtained, as shown in tables and figures. Especially, methanol and ethanol of 0.05% were effective as activators in many cases.

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Micrococcus glutamicus の細胞学的研究

第2報 電子顕微鏡的形態ならびに構造観察および極顆粒について

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Shiro ITAGAKI* and Shukuo KINOSHITA*: Cytological Studies on *Micrococcus glutamicus*. Part II. Electron-Microscopic Observations on the Morphology and Construction of the Cell, and on the Polar Granule.

1958 年 10 月 18 日受付

Micrococcus glutamicus について，第1報[1]では主として methylene blue 染色および cell wall 染色をして経時的形態変化を観察し，その主因たる不完全分裂が biotin の不足によって起こされることを明らかにし，またとくに極在性の小顆粒の存在を認め，その消長についてのべ，あわせてその核分裂様式を観察して無糸分裂によることを示した。

本報では，電子顕微鏡による観察，とくに超薄切片法による観察をおこなって本菌の菌体構造について若干の知見をえ，さらに極顆粒がいわゆるvolutin granule に一致するものであろうことを明らかにしたので，ここに報告する。

実験材料および実験方法

使用菌株，培地および実験方法などは第1報でくわしくのべたので省略する。超薄切片法についてはその項でのべる。

結　　果

1. 極顆粒について

第1報において極顆粒と称したものは Neisser の染色によりいわゆる volutin granule に一致することが明らかとなった。 volutin granule, あるいは metachromatic granule は *Corynebacterium diphtheriae* において特異的といわれるほど明瞭に出現する小体であるが，その化学成分，生物学的意義などはまだじゅうぶんに明らかにされてはいない。大体，polyphosphate を主体とするエネルギーのプールであると考えられているようだが，その成分は菌株によって差異もあり，じゅうぶんな研究がなされているとはいいがたい。

2. 電子顕微鏡による経時的形態観察

a） glucose bouillon 培養

電子顕微鏡観察（以下EM観察と略す）によれば，glucose bouillon 培養においてもおそらく volutin granule と考えられる顆粒の存在は明らかである。この時期の顆粒の大きさは約 100～150 mμ であり，methylene blue 染色によってその存在が認められなかったのは，このようにきわめて小さいためであろう（光顕の分解能以下)。

菌体の大きさは，glucose bouillon ではおよそ 0.5～0.8×1～1.5 μ ぐらいのやや楕円形を呈する小球菌形を示す。この数値は光顕による場合よりやや小さい。

b） 合成培地

1.5～7^h の間は volutin granule はきわめて明瞭である。その大きさは 300～400 mμ に増大する。このことは，この時期で光学顕微鏡観察が容易になった一因である。

9～12^h においてもなお EM 観察で容易に認められる。

methylene blue 染色で認められた中央濃染部，すなわち，核に相当すると考えられる部分は，electron opaque 部分として観察され，12^h ぐらいまでの菌体に明瞭に認められ，分裂像と考えられる所見も多数えられた。これらはみな写真で

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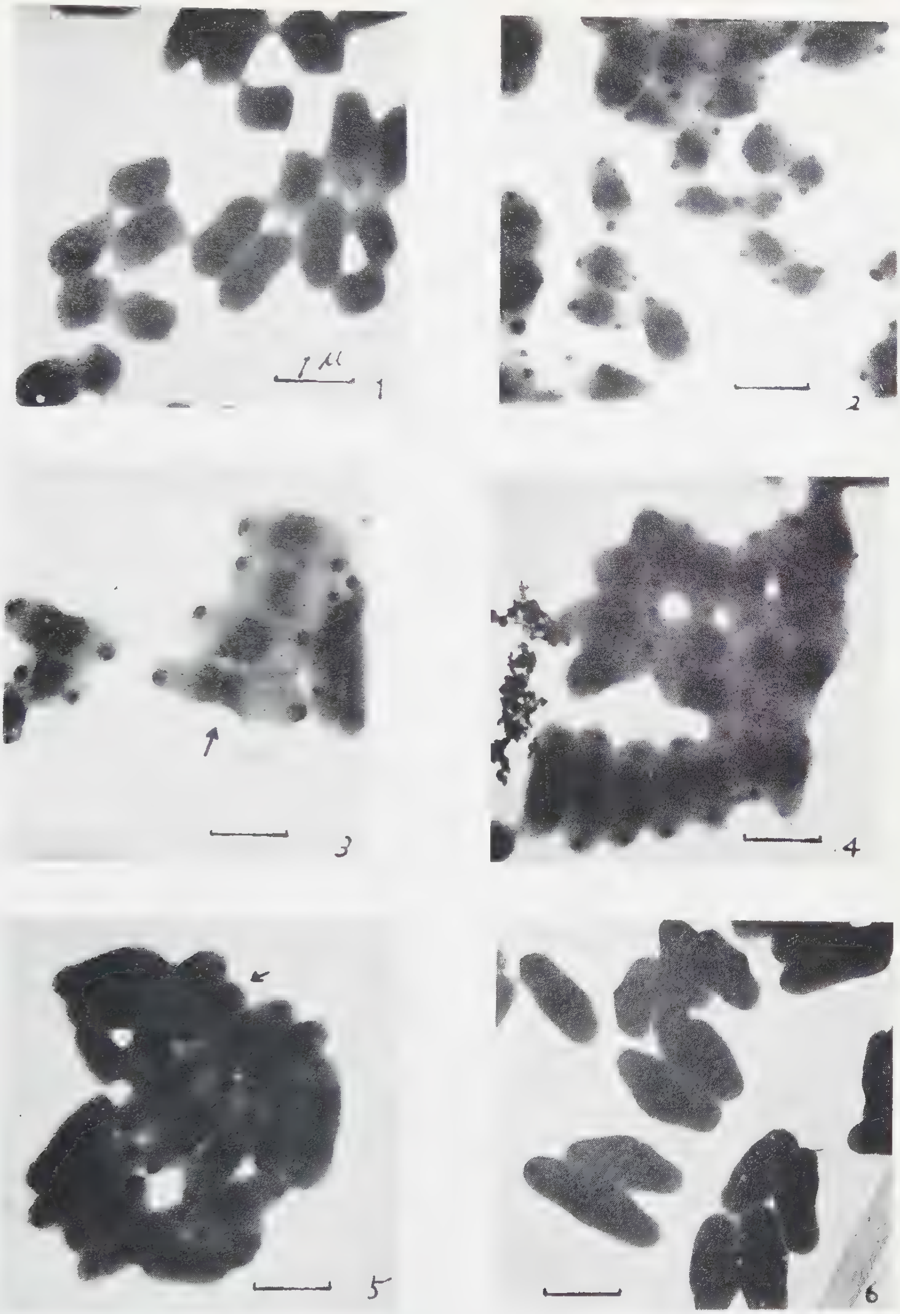
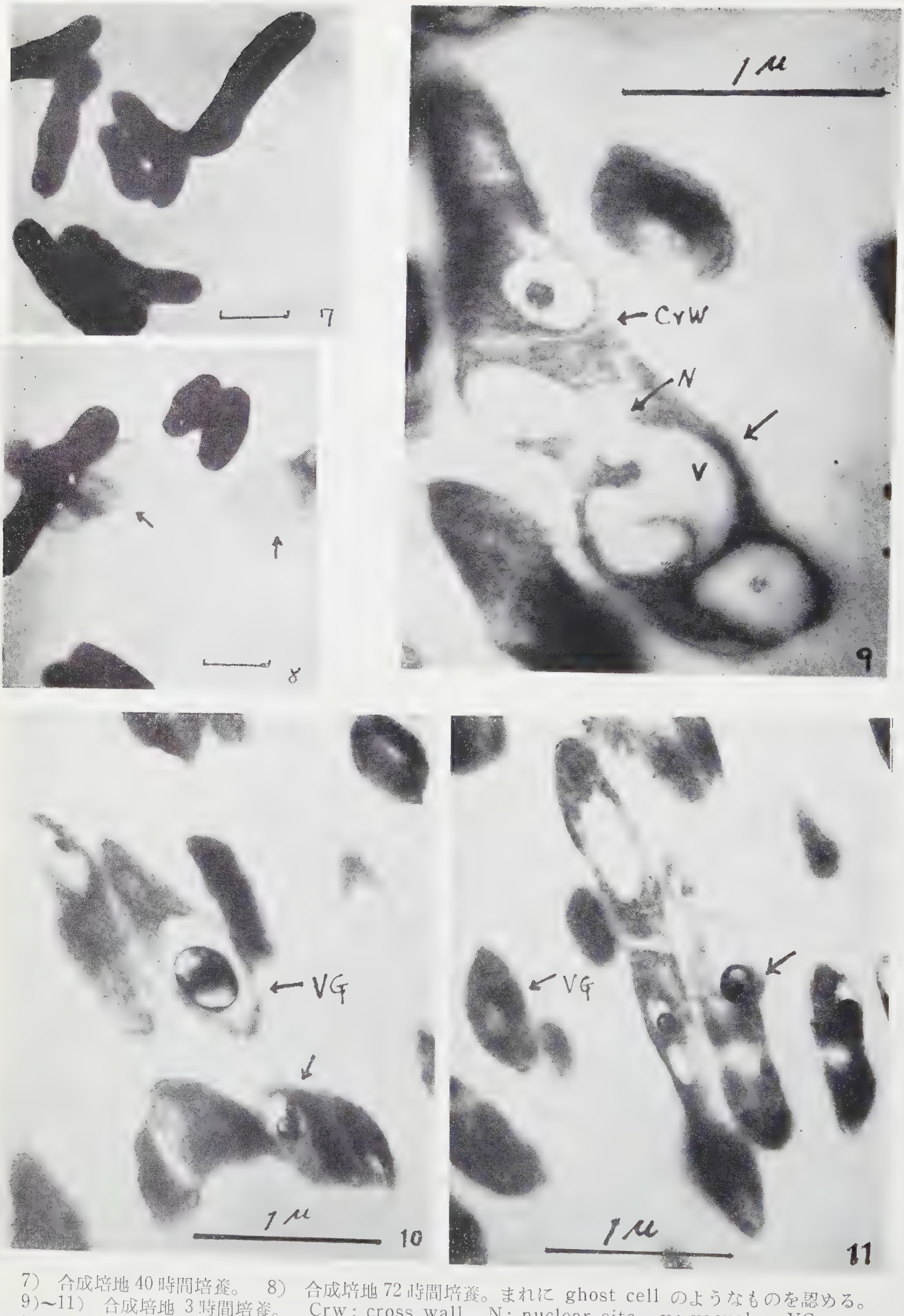


1) glucose bouillon で24時間培養したもの。 volutin granule がきわめて小さいことに注意。 2) 合成培地で1.5時間培養。volutin granule に大小2種類存在することが見られる、合成培地で volutin granule が大きくなる過程であろう。 3) 合成培地5時間培養，細胞分裂の各時期に注意 鮮明な volutin granule が認められる， 4) 合成培地9時間培養。 5) 合成培地 12 時間培養。細胞質の密度がいくぶん高くなってくる。vacuole のようなものが認められた。 6) 合成培地 24時間培養。volutin granule を認めることはできない。細胞質の密度は高い



7） 合成培地 40 時間培養。 8） 合成培地 72 時間培養。まれに ghost cell のようなものを認める。9)～11） 合成培地 3 時間培養。 Crw: cross wall, N: nuclear site, v: vacuole, VG: volutin granule volutin granule の小胞構造に注意。（スケールはいずれも 1μ を示す。）

示してある

20^{h} 以降，菌の形態はすでに methylene blue 染色で見たように不整であり，長桿形を呈している まれに崩壊像のような所見もあるが，一般に 72^{h} までの観察で電子密度はきわめて高くなり，内部構造の観察は行ないえなかった。なお 56～72^{h} において ghost cell のようなものを認めた。合成培地では培養が進むにつれて菌体は長くかつ肥大し，12^{h} で 0.7～1.0×1.5～3.0 μ ぐらいになり，72^{h} では長さ 4.0 μ ぐらいに達する場合があるが，一般には 1.0×2.0 μ ていどの値を示す。

使用した電子顕微鏡は，日本電子光学研究所製 JEM-T4 型である。

3. 超薄切片法による菌体構造の観察

超薄切片法がEM観察の手段として登場していらい，細菌学の領域でも新しい有力な研究方法として採用され，すでに幾多の業績がなされているが，ここではその紹介は省略する。

実験方法としては東[2] の方法にしたがった す なわち，合成培地で 3^{h} 培養した菌を遠沈して集菌後，pH 7.2 の phosph. buffer で数回洗滌，2% OsO_4 水溶液を用い，最終濃度が約0.7%になるように phosph. buffer 懸濁液として固定した。固定時間は室温にて 5，10 および 22^{h} とした。

使用したミクロトームは，日本電子光学研究所製 JUM-3 型である。

cell wall の厚さは 10 mμ 以下で，密度は細胞質よりやや低いように思われる。

細胞質はややあらい構造を呈し，菌体中には異常に大きな液胞を認めることが多い その数は多い場合には 3 個，概して 1～2 個であり，その中にきわめて electron dense な顆粒が存在する。細胞質中，やや密度の低い部分が，菌体のほぼ中央に存在するが，この部分は核染色陽性部位と一致することからみて，いわゆる nuclear site であろうと考えられる。しかし，多くの細菌において認められたラセン糸に相当する紐状構造は認められず，何となく fibrous な構造という程度である。

本菌における顆粒，すなわち，volutin granule の出現・消長は培養を経時的に追跡して，興味ある結果を得たことはすでに第 1 報[1] で述べた。本顆粒の密度はきわめて高く，しかもその構造は数十の小胞に分かれているもようである。小胞は 5mμ 以下の場合もあり，大きい場合はまれに 150 mμ に達するが，概して 5～20 mμ ぐらいである。この構造は武谷等[3] が結核菌において認めたような意味の artifact とはやや異なるのではないかと考える この点については後に論ずる。

考　　察

第 1 報[1] で極顆粒と称したものは，Neisser 染色によれば，いわゆる volutin granule あるいは metachromatic granule であると推定される。従来この granule については相当多数の研究がなされており，その菌体内における形成[3,4,5,8]，化学的組成等[4,7,8,9,10,11,12]，が論ぜられてはいるが，現在では polyphosphate を主体としたエネルギーのプールであろうと考えられている。しかしその生物学的意義はなお不明に近いといえよう。

著者等はこの顆粒の構造に関し，前述のように超薄切片法によって観察した結果，5～20mμ の単位小胞とでもいうべきものの集合により構成されているのではないかと云うことをみた。このことは，本顆粒を，栄養物質を単に貯蔵する場だとする見解では不適当なことを感じさせる。武谷[4] は結核菌のEM観察にあたって，いわゆるA顆粒に強い beam を照射すると，菊花状，あるいはドーナッツ状の変化をみせるとのべている。*M. glutamicus* の場合，強い beam の照射に対してもなんらの変化も認められない 著者等の小胞構造はこのような意味での人為像ではないと考えている。

Chapman および Kroll[13] は，*Spirillum serpens* の超薄切片法による研究において，細胞質中に一種の顆粒を認め，high density particle とよんでいるが，この所見は比較的著者等の観察に近い しかし彼等はたまたま 1 個の顆粒を観察したにすぎず，多分人為像であろうとのべている。著者等の場合はほとんど全菌体に規則的に存在することより見ても明らかに人為像ではない。

しかしここで問題になるのは培養時期にしたがって認められなくなること，むしろ存在を確認できる時期はきわめて短いことである。これははたして本当に消失するのか，あるいはすでにのべたような方法では観察しえなくなるのであるかが，

明らかでない。この点を明確にするためにはさらに培養後期の菌体を超薄切片法で観察するよりほかはないと思われる。24h 培養菌体について行なった結果は，この顆粒の存在を確認することができなかったが，じゆうぶんな実験とは云いがたいので，この点は保留し今後さらに検討したい。

著者等は別にこの顆粒に酷似する顆粒を *Aspergillus orizae* の conidia中に，認めて報告した[14]。

要　約

Micrococcus glutamicus について，主として電子顕微鏡観察をおこない，二，三の知見をえた。

1. 培養初期に認められる極顆粒は，いわゆる volutin granule あるいは metachromatic granule にほぼ一致することを明らかにした。
2. glucose bouillon 培養でもきわめて小さい volutin granule を認めた。大きさは 100～150 mμ 程度である。
3. glucose bouillon 培養では菌体の大きさは，およそ 0.7～1.0×1.5～3.0 μ である。
4. 合成培地における菌体の大きさはおよそ 1.0×2.0 μ ぐらいに増大し，いちじるしく電子密度を高める。
5. volutin granule は一般に合成培地において培養された場合，1.5～7.0h の間に300～400 mμ の大きさに達する。
6. cell wall の厚さは 10 mμ 以下であり，密度は細胞質よりやや低い。
7. 細胞質はややあらく，時にきわめて大きい液胞を含む。
8. 細胞質中とくに密度の低い部分が認められ，やや fibrous な構造を示すが，この部位は nuclear site に一致すると考えられる。
9. 液胞は通常 1～2 個であるが，その中にきわめて電子密度の高い volutin granule を1個含む。
10. volutin granule は 200～400 mμ くらいの大きさになるが，これはさらに 5～20 mμ くらいの多数の小胞に分かれている。

いろいろ御助言をいただいた，東大教授湯浅明博士，慈恵医大教授富井武寛博士，慶応大学助教授渡辺陽之輔博士に深く感謝する。

附記：本研究の第1報と第2報で用いた菌株は，特許公報，昭和 32-8698 記載のものと同一である。

Summary

Electron-microscopic observations were made on *M. glutamicus*.

1. Polar granules which appear at some stages of incubation are thought to be volutin or metachromatic granules.

2. Very small volutin granules were also observed in the cells which were cultured in glucose bouillon. Size of these granules were 100 mμ-150 mμ in diameter.

3. Size of volutin granules increased to 300 mμ-400 mμ in diameter after 1.5h～7h culture in the synthetic medium.

4. The thickness of the cell wall is less than 10 mμ and its density is lower than that of cytoplasm.

5. There are a few large vacuoles in cytoplasm.

6. Low density part which was slightly fibrous was observed in cytoplasm, and may be considered to be the nuclear site.

7. Usually, there are one to two vacuoles which contain a volutin granule of high electron density.

8. Volutin granule is consisted of many small vesicles whose diameters are 5 mμ-20 mμ.

文 献

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四国の亜高山帯森林植生について

山　中　二　男*

Tsugiwo YAMANAKA*: On the Subalpine Forest Vegetation in Shikoku, Japan

1958 年 12 月 1 日受付

わが国の亜高山帯森林植生は，本州中部地方以北にひろく発達し，南は紀伊半島の一部にあらわれ，さらに四国山脈に及んでいる。本州の亜高山帯森林植生については，各地で群落学的研究がおこなわれているが，四国地方のそれも山中[1]，佐藤[2]，山中[3]，その他によって，その概要が報告されてきた。四国地方の亜高山帯の植生は，その地域が局部的で，また発達の規模も小さいが，現在のわが国においては注目すべきものもあり，いろいろ興味ある問題を有すると思われる。筆者は，四国地方の森林植生の調査の一部として，亜高山帯の植生をも調査する機会を得たので，今までに得られた資料を整理して，ここに総括的に記述考察したいと思う。

調査地と方法

四国地方で亜高山帯森林植生のみられる徳島県剣山，愛媛県笹が峰および石鎚山で森林植生の垂直的変化を観察し，組成について方形区法によって調査をおこなった。方形区の大きさは 10×10m. とし，Braun-Blanquet[4]の方法におおむね準拠して，フロラ組成と優占度を判定し，それによって綜合優占度と恒存度を求め，第 1 表にかかげる結果を得た。このような組成の資料と，さらに気候，地形，土壌などの環境との関係を検討し，四国の亜高山帯森林植生を生態学的に明らかにするようこころみた。

組成と相観

四国地方の亜高山帯森林植生の優占種は，シコクシラベ（*Abies sikokiana* Nakai＝*A. veitchii* var. *sikokiana* Kusaka）である。このシコクシラベ林は，後述するように主として上記三山の 1700〜1800m. 以上によく発達するが，この地域内でも，おそらく二次的に生じたと思われるササ原が，相当に広範な地に見られ，またところによりダケカンバ（シコクダケカンバ *Betula shikokiana* Nakai とよばれたもの）の林が存在し，代表的なシコクシラベ林の見られる範囲はかなり限られていて，本州中部以北の亜高山帯針葉樹林に比しフロラ組成もいくぶん単純な傾向がある。

高木層にはシコクシラベが優占するが，ときにダケカンバ，ヒメコマツ，ナナカマドなどが混生し，ことに発達初期の林や岩石地ではダケカンバの優占度が大きい。シコクシラベの樹高は多くの場合 6〜10 m. 胸高直径は 10〜40cm. のものが多いが，風衝のはげしい石鎚山の頂上近くのようにほとんど低木状態にとどまっているところもあり，概して伸びのよくないところが多い。低木層は主としてササであり，コヨウラクツツジ，その他おもにツツジ科の植物をともなう。このササは樹冠の密接した老令林あるいは岩石地の林では，優占度が小さくなり，あるいは矮小化し，ときにまったく欠如するにいたる。草本層にはコミヤマカタバミが普遍的に多く，この林を四国の他の植物群落から区別するに役立ち，その他シラネワラビ，カニコウモリ，マイヅルソウ，ヒナスゲ，ショウジョウスゲ，ハリブキなどの多く生ずるところもあり，またツタウルシがひろく匍匐するなど，林床のフロラは場所により多少の差違がある。また低木層にササの優占するところほど，草本層の発達は貧弱であるが，ササの少ないところでは草本層

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の構成植物が多い。林床の蘚苔類は，ことにササを欠くところではよく発[illegible]，[illegible]型というべきもので，*Hylocomium splendens*, *Pleurozium schreberi* などがことに優占するが，ほかに *Polytrichum formosum*, *Dicranum majus*, *Hypnum fujiyamae* なども多い。

このように，組成的には場所によりいくぶん異なり，いくつかの基群集がみとめられるが，相観的には高木層は *Abies* type で，ときに *Betula* type がまじる。林床は *Sasa* type, *Oxalis* type, *Hylocomium* type が代表的である。このような林床と相観の点は，[illegible]とおなじであるが，四国地方ではヤマソテツの多い plagiogyria type というのは見られない。

考　　察

シコクシラベ林は，剣山と笹ヶ峰ではおおむね 1800 m. 以上によく発達し，石鎚山ではやや低く 1700 m. からすでにはっきりした林となって存在している。[illegible]にも見られ，[illegible]でも 1700 m. 内外まで下降して生育してはいるが，しかしこのような高さでは，すでにはっきりしたシコクシラベ林としての姿はうすれ，温帯の極盛相であるブナ-スズタケ群集が優占[illegible]。また[illegible]のみからみれば，他に 1800m. 以上でもシコクシラベ林の見られない山があるが[illegible]，[illegible]から四国地方の亜高山帯植生は，少なくとも1700～1800 m. 以上でなければ存在しないと考えられる。

地質は剣山の上部は古生層で，山容はおだやかである。笹ヶ峰は結晶片岩，石鎚山は安山岩質のもので，地形は剣山に比し急峻である。これらの母岩は，直接には植生の発達とは大きな関係をもたないようであるが，地形的に急傾斜の岩石地などでは，垂直分布にある程度の差があらわれ，かような場所では亜高山帯の森林が多少下降してゆく傾向があるようである。

気候は環境条件としてはもっとも重要である。剣山頂測候所の観測資料によると，年平均気温は 4.1° で，最低月(1,2月)は−7.4°，最高月(8月)は 15.2° であり，暖かさの指数は 36.1°，寒さの指数は−46.2°となる。暖かさの指数 45° が温帯の落葉広葉樹林の上限と考えると[6]，石鎚山，剣山ともに 1700 m. で 43° 内外と推定されるから*，この高さではすでに亜高山帯針葉樹林に入らねばならない。しかし[illegible]その他を考慮に入れると温量指数による推定は，四国地方でも大[illegible]と考えられる。降水量は剣山では 4～10月の 7 ヶ月間で 2300 mm. 以上であり，ことに 6～10月は毎月 300 mm. をこしている。11～3月は[illegible]が多く，くわしいことがわからないが，[illegible]も太平洋型のものであることには間違いない。

四国の温帯林はブナ-スズタケ群集[7]を極盛相とし，このブナ林上部で亜高山帯の直下には，しばしばウラジロモミの群落がでてきて，本州でよく見られるコメツガ林は全然ないわけではないが[4]，欠いているところが多い。石鎚山附近では約 1700 m. 内外まではウラジロモミ林が存在していて，この上部のシコクシラベ林とはかなりはっきり交替している。またシコクシラベ林の存在しない 1800 m. 以上の山岳では，上部までウラジロモミが見られる。このような点から考えると，四国ではウラジロモミの多い林が，多くの場合温帯林の上限となって，その上部にシコクシラベ林の出現をみるということが普通とみなされる。土地的にウラジロモミが亜高山帯の領域内に入ると思われることもあるが[4]，全般的には今西[9]の太平洋型の[illegible]がみとめられる。ただ前述のごとく，亜高山帯[illegible][10]，[illegible][11]，中部地方[illegible]のようなコメツガ林の発達が四国ではほとんど見られないことは注意しなければならない。なおダケカンバ林は，剣，石鎚両山地の上部で各地に見られるが，多くは二次林として存在し，またしばしば落葉広葉樹林帯まで下降し，これを亜高山帯の指標としてえらぶことには無理がある[4),10),11),13),14)]。

シコクシラベ林の組成は既述のようであるが，この林床群落の相違は土地条件と光によるものが多いと思われる。壮令林の多くはササ型の林床をもっているが，これとイワダレゴケ型の林床は，土地的にかなり明らかに異なっている。すなわち，地盤の安定した比較的深土のところではササ型であるが，基岩の露出の多い不安定な場所では

* 剣山は山頂の資料から，石鎚山は松山の資料から，それぞれ減率 0.47 と 0.55 をもって計算した[8]。

Table 1. Association table

Locality	Mt. Tsurugi					Mt. Tsurugi					Mt. Ishidzuchi				
Altitude (m.)	1900–20					1900–20					1970				
Exposition	S25E					S05E					S75W				
Steepness	35					20					45				
Tree height (m.)	6-8					8-10					3-8				
Tree layer															
Abies sikokiana	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5
Betula ermani	4	3	3	3	1	—	—	—	—	—	—	—	—	—	—
Sorbus commixta	—		(+)	(+)	(+)			—				+		—	
Pinus pentaphylla v. *himekomatsu*	—	—	—	—	—	2	—	1	2	2	3	2	2	—	—
Schizophragma hydrangeoides	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tripterygium regelii	+	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Shrub layer															
Sasa spp.	5	5	3	+	5	5	5	5	5	5	5	5	5	5	5
Menziesia pentandra	3	2	3	3	—	—	—	—	—	—	—	—	—	—	—
Tritomodon cernuum	—	—	—	—	—	—	—	—	—	—	1	2	—	—	2
Viburnum urceolatum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rhododendron pentaphyllum	—	—	—	—	—	—	—	—	—	—	1	1	1	—	+
Rhododendron metternichii	—	—	—	—	—	—	—	—	—	—	+	—	1	—	—
Viburnum furcatum	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
Herb layer															
Oxalis acetosella	2	2	3	1	1	+	2	2	—	+	—	—	—	—	—
Dryopteris austriaca	3	2	3	4	—	—	—	—	—	—	—	—	—	—	—
Rhus ambigua	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carex grallatoria	—	—	—	—	—	—	—	2	—	—	+	+	—	—	—
Majanthemum dilatatum v. *nipponicum*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Cacalia adenostyloides	—	+	2	3	—	—	—	—	—	—	—	—	—	—	—
Carex blepharicarpa	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—
Rumohra mutica	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Oplopanax horridus v. *japonicus*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Galium kamtschaticum v. *acutifolium*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Luzula rostrata	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Circaea alpina	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Acer micranthum	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—
Rubus pseudo-acer	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—
Hugeria japonica v. *ciliaris*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lycopodium serratum v. *thunbergii*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Phegopteris polypodioides	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vaccinium hirtum	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lastrea japonica	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Coptis quinquefolia	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Paris tetraphylla	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lycopodium clavatum v. *nipponicum*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Brylkinia schmidtii	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Acer sieboldianum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+

of Abietum sikokianae

Mt. Sasagamine 1800 S60E 15 6-8 (−10)					Mt. Ishidzuchi 1750-60 S80E 30 6-10					Mt. Ishidzuchi 1845-55 N25W 30 6-10					Mt. Tsurugi 1860 S15E 20 5-8					Coverage value	Constancy
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	8678.6	X
{+}	—	{+}	{+}	—	—	—	—	—	—	—	—	2	3	—	—	—	—	-	—	836.5	III
{+}	—	{+}	{+}	{+}	{+}	{+}	{+}	{+}	{+}	+	3	1	+	1	2	2	(+)	1	(+)	255.1	VII
·	-	—		—	—		—	1	—	—	—	—	—	—	—	—	—	—	—	385.7	III
—	—	·	—	—	—	—	—	—	—	—	—	2	{+}	—	—	—	—	—	—	50.3	I
{+}	—	{+}	—	—	{+}	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15.5	II
4	4	4	4	4	5	5	5	5	5	(2)	(1)	—	(2)	(1)	—	—	—	—	—	5729.0	VII
—	—	—	—	—			-	-	-	—	—	+	—	—	3	2	4	2	3	864.7	IV
·	—	—	—		-	-	-	-	—	—	—	—	—	—	—	—	—	—	—	114.3	I
—	—	—	—	—	·	-		—	—	-	—	·	—	·	1	2	1	1	1	107.2	II
—	—	—		-	—	—	-		—	—	—	—	—	—	—	—	—	—	—	43.2	II
·	—	·	—	—		·	—	·	—	—	—	—	—	—	—	—	—	—	—	14.6	I
—	—	—	—	—	-	—	—	-	—	—	—	—	—	—	—	—	—	—	—	14.3	I
—	2	2	—	—	—	—	—	—	—	3	2	3	3	2	4	5	4	4	4	1971.4	VI
—	2	1	—	—	—	—	—	—	—	+	+	—	1	+	3	3	3	3	2	999.7	V
—	—	—	—	+	+	3	1	2	1	—	—	—	+	+	4	3	3	4	3	865.4	IV
+	2	—	+	+	—	1	+	—	1	—	—	3	3	3	—	—	+	—	—	466.3	V
—	—	—	—	—	—	—	—	—	—	3	2	1	—	—	—	—	—	—	—	171.4	I
—	—	—	—						—										—	157.4	I
—	—	—	—	—		—	·			1	2	1	—	—	—	—	—	—	—	79.2	II
—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	28.6	I
—	—	—	—	—	—	—	—	—	—	+	+	+	—	1	—	—	—	—	—	15.2	II
—	—	+	—	+	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	14.9	I
+	+	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	14.9	I
—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	14.3	I
—	—	—	—	—		-	·	·	-			[illegible]	[illegible]			·			—	1.2	II
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	0·6	I
—	—	—	—	—	—	+	—	—	—	—	+	—	—	—	—	—	—	—	—	0.6	I
—	—	—	—	—	—	—	—	—	—	—	—	+	+	—		—		—		0.6	I
—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—	0.6	I
—	—	—	—	—	—	—	—	—	—	·		—	·		·	—		—	—	0.3	I
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.3	I
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-	—	-	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.3	I
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—	—	—	—	—	—	—	—	—	—	—	—	—	—			—		·		0.3	I
—	—	—	—	—	—	—	—	+	—	—	—	—				—	—		-	0.3	I

イワダレゴケ型である。また遷移のうえから見れば，林が老令になるとササ型はイワダレゴケ型に変わってゆくことがみとめられる。しかし，イワダレゴケ型はササの少ない岩石地などでは初期林から見られ，かような場所ではササの密生による稚樹の生育阻害はなく，その数はきわめて多く，25 m.² 内に 70～80 本内外が見られるが，樹冠の密閉による受光量の不足と粗腐植の堆積地では，稚樹の生長は緩慢である。森林の疎開により後継樹はよく生育し，ために石鎚山面河斜面に見られるごとく，イワダレゴケをもつシコクシラベ林が，むしろ土地条件のわるいところに発達し，しかも下方に林が伸びる傾向が見られる。

シコクシラベ林内のササは，ブナ林上部のものと共通である。これらは分類学的には問題を残しているが，生態学的なことも考えるとチシマザサ系統のものではない。この点はブナーチシマザサ群集とブナースズタケ群集が異なるように，日本海型の亜高山帯の森林と太平洋型のそれとは異なっている。中部地方に多いシラベ，トウヒなどの林は，アオモリトドマツをまじえることも多く，前田[10),12)]は秩父山地，木曽御嶽の林をアオモリトドマツーシラベ群集としている。しかし，太平洋型のシラベを主とした林を，八甲田山[15)]や尾瀬地方[16)]でよく研究された日本海型のアオモリトドマツ群集から区別して考える立場は，十分意味があると思われる。四国のシコクシラベ林は，本州のシラベ林からトウヒやアオモリトドマツを欠き，コメツガもほとんどまじえず，高木樹種は単純となっているが，一方ダケカンバ，ナナカマド，コヨウラクツツジ，オオカメノキ，コミヤマカタバミ，カニコウモリ，シラネワラビ，シノブカグマ，ハリブキ，マイヅルソウなど多くの主要な構成植物が共通している点では，同一群集に入るものとの見方もできると思われる。しかし，組成的な相違がないわけではなく，また本州におけるシラベ群集という名称と性格が，現在はっきりしていないため，ここではシコクシラベ群集 (*Abietum shikokianae*) と仮称しておくことにする。この群集はアオモリトドマツやシラベの林とともに，わが国亜高山帯針葉樹林として一の群団をなし，*Vaccinio-Piceetea*[17)] の一部をなすものである。

要　約

四国地方では，剣山，笹ケ峰，石鎚山にシコクシラベを優占種とした亜高山帯針葉樹林が見られる。この林の発達は局部的で，規模も小さいが，代表的なところでは，高木層にシコクシラベが優占し，林床はササ型あるいはイワダレゴケ型が多い。組成的には本州のシラベ林と共通するところが多く，同一群集とも考えられるが，ここではシコクシラベ群集と仮称した。このシコクシラベ群集は，上記三山の海抜 1700～1800 m. 以上に発達しているが，四国の亜高山帯では，シコクシラベ群集の下部にコメツガ林はほとんど発達せず，一般にウラジロモミ林またはブナ林とシコクシラベ群集がつらなっている。

この研究に御指導をたまわった広島大学堀川教授，種々有益な御教示をいただいた大分大学鈴木教授，蘚類の同定を願った熊本大学野口教授，その他現地調査に援助していただいた各位に心から感謝の意をあらわすしだいである。

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* たとえば，石鎚山地ではイシヅチザサ (*Sasa ishizuchiensis* Makino があり，剣山ではシコクザサ (*Sasa hirtella* Nakai がある。

Summary

Subalpine coniferous forests mainly dominated by *Abies mariesii* and *A. veitchii* develop widely in the northern and middle Honshû. In Shikoku, however, the subalpine region is limited, and the climax forest occurs on a rather small scale. The mountains where the subalpine coniferous forest is found in Shikoku are Mt. Tsurugi, Mt. Sasagamine and Mt. Ishidzuchi, and the climax forest of the subalpine region is characterized by the dominance of *Abies sikokiana* (*A. veitchii* var. *sikokiana*).

The floristic composition and the structure of this *Abies sikokiana* forest are presented in Table 1. *Abies sikokiana* dominates in the tree layer usually attaining to 6–10 m. in height. *Betula ermani, Pinus pentaphylla* var. *himekomatsu*, and *Sorbus commixta* occur frequently but are not always abundant. The shrub layer is constituted by *Sasa* spp., *Menziesia pentandra*, and others, but *Sasa* spp. are mostly dominant. The major species in the herb layer are *Oxalis acetosella, Dryopteris austriaca, Cacalia adenostyloides, Majanthemum dilatatum* var. *nipponicum*, and *Oplopanax horridus* var. *japonicus*. The forest-floor, where *Sasa* spp. are absent or are not abundant, is predominated by mosses such as *Hylocomium splendens, Pleurozium schreberi*, etc.

The floristic composition mentioned above has distinct resemblances to that of the *Abies veitchii* forest occurring in the Pacific side of Honshû. Though the forests of *Abies veitchii* and *A. sikokiana* may be included in one and the same association, a provisional name, the *Abietum sikokianae*, is given here.

Abies sikokiana often comes down to an elevation of about 1500 m. above sea-level. But the climax subalpine coniferous forest is of course not found in such an altitude, and *Fagus crenata* is still predominating. It is assumed that the area of the *Abies sikokiana* climax forest is at least higher than 1700 m. altitude. The lower limit of this forest usually comes in contact with the *Abies homolepis* forest or the *Fagus crenata* forest, and the *Tsuga diversifolia* forest is seldom found in the lower part of the subalpine region of Shikoku.

第9回国際植物学大会

ことしの8月19日から29日までの11日間カナダ国 Montreal で第9回国際植物学大会がひらかれる。会場は McGill University, University of Montreal, Sir George Williams College であり，なおこの学会にさきだって8月16日から19日まで植物命名規約についての議事がおこなわれることになっている。第一回の案内状が去年の6月に世界中に発送されたが，それに対して出席の通知を学会組織局によこした人は約6000人に達するという。第8回の国際植物学大会は1954年にパリでひらかれたが，今回は慣例に反してそれからかぞえて5年めになる。6000人という人数は，多分いくらかへるであろうが，ともかくかなりな盛会になるとおもわれる。

学会における部のかずはパリのときよりいくらかすくないが，組織局の努力によって植物学のいろいろな研究分野ごとに部 (section) がつくられ，シンポウジアム，討論をする時間がもてるように注意してあり，それとともに個人的に出席者が接触できるように考慮されている。講演は午前は9時から12時まで，午後は2時から4時まで，招待講演と公開講演とは夕刻からおこなわれるようになっている。

次に公開講演，招待講演，シンポウジアムそのほかめぼしい演題をしるしておく。

A．総会シンポウジアム

1. 花とコンチュウとの関係の進化（ダーウィン100年記念シンポウジアム） V. I. Grant
2. 植生の生産性 G. E. Hutchinson

B．公開講演

1. ダーウィン100年記念講演(フランス語) Roger de Virmorin
2. ダーウィン100年記念講演(英語) Paul B. Sears
3. 植物生理学と，全体としてみた植物の問題（フランス語） A. Kursanov
4. 植物学と人生（英語） E. C. Stakman

C．総会講演

1. カナダの植生（フランス語） Pierre Dansereau
2. カナダの北極および亜北極地方の探検（英語） Jacques Rousseau
3. 幻想をひきおこすキノコ（フランス語） Roger Heim
4. 未定

D．部の組織

第1部 命名

この部についての案内は "Taxon" にでている。

第2部 一般分類学(特殊な問題をふくむ)と系統学

この部には次のシンポウジアムと展示とがくみこまれている。

1. 分類の体系 A. Cronquist
2. 分類学的問題と植物の比較化学 講演者は未定
3. 分類学に解剖学を応用することについて 講演者は未定
4. イネ科の自然分類 J. Reeder
5. 分類学と発生学 P. Maheshvari

展示 菌学者の見解からみたユキノシタ科の進化 D. B. O. Savile

第3部 藻植学シンポウジアム

1. 藻類系統についての最近の所見 G. F. Papenfuss
2. 藻類培養の最近の進歩 E. G. Pringsheim
3. 海藻の生態学 J. Feldmann
4. 淡水産藻類の生態学 P. Bourrelly
5. 植物における鞭毛をそなえた可動性細胞 J. Couch

第4部 菌学シンポウジアム

(一般菌学)

1. Hymenomycetes の分類と目，科，属の近代的概念 A. H. Smith
2. 子のう菌類の完全な状態と不完全な状態とのあいだの相互関係とその分類に対する関係 E. W. Mason
3. 下等菌類における分化 Ralph Emerson
4. 菌類の形態形成に影響する栄養因子 Lilian Hawker

5. 菌類の生産物を変化させる手段としての heterokaryosis と para-sexuality Kenneth B. Raper
6. 藻類学における motile flagellated cells J. Couchの講演

講 演

1. 日本の担子菌類 今井三子
2. 菌類の生きた核における観察

展 示

Polyporaceae の分類に関連した培養 M. K. Nobles

医 学 的 菌 学

1. 宿主と寄生植物との関係と分類学におけるその意義
2. 病原となる菌類の源泉としての土壌 C. W. Emmons

講 演

1. 北アメリカにおける blastomycosis N. F. Conant
2. Histoplasmosis M. L. Furcolow

第 5 部 植物病理学

シンポウジアム

1. 宿主と寄生植物との関係の生理学
2. 植物ビールス対策における進歩 R. E. Fitzpatrick
3. アリマキによる植物ビールスの転移 M. A. Watson
4. 植物に対する寄生ネマトーダの関係 E. J. Cairus
5. 寄生現象の解釈と実験遺伝学 T. Johnson
6. 植物における腫瘍の形成の機作 A. C. Braun
7. 越冬する植物に対する耐寒性菌類の寄生 J. G. Dickson
8. 根の損壊の病理 L. W. Koch
9. サビ病における分化と宿主寄生植物間の関係 E. C. Stakman
10. 植物の病害対策 A. E. Dimond
11. 耐病性の性質と遺伝 J. C. Walker

第 6 部 蘚苔学

シンポウジアム

上にあげた J. Couch の話題

第 7 部 微生物学

シンポウジアム

1: 植物の生長に対する微生物の関係 —根圏— R. L. Starkey
2. 土壌における協同的および対抗的現象—抗生物質, 生長促進物質 D. Gottlieb
3. 微生物および高等植物による抗生物質の生成
4. 植物病病原としての細菌のメタボリズム, 栄養および遺伝 E. D. Garber
5. 窒素固定 A. I. Virtanen

第 8 部 形態学, 解剖学

発生学の部 シンポウジアム

1. 顕花植物における Apomixis と多胚性 E. Battaglia
2. 前記 Maheshwari の話題
3. 顕花植物における配偶体, 胚, 胚乳の関係 M. Cave

形態学, 解剖学の部

1. 超顕微鏡的細胞形態学 K. Muhlethaler
2. 葉条の新生組織 E. Ball
3. 葉条とその付属物 C. W. Wardlaw
4. 根とその付属物 J. C. Torrey
5. 分化の解析 D. S. Van Fleet
6. 顕花植物の起原と進化 A. J. Eames
7. 分類学と解剖学

第 9 部 維管束植物の分類と地理学

シンポウジアム

1. 植物区景の筆者の直面する実際的問題
2. 高山植物区景と北極植物区景
3. シダ類の分類についての問題
4. 生物分類 (Biosystematics)

第 10 部 古植物学

シンポウジアム

1. Tracheophyta の系統学における進化 A. J. Eames
2. 形態学的性質と植物区景構成とに対する指標としての微生物化石 (午前) C. A. Arnold (午後) R. M. Kosanke

第 11 部 生理学

1. 植物細胞とその生理学的および構造的体制
2. 細胞の形態形成 B. Brown
3. 植物体内における水のストレス H. Oppenheimer

4. イオンの吸収と転移における原形質のやくわり W. H. Arisz
5. *in vivo* および *in vitro* におけるクロロフィルの光化学と吸収スペクトル C. S. French
6. 光化学反応につづく光合成の生化学 M. Gibbs
7. エネルギー発生と共役エネルギー転移 D. Goddard
8. 高等植物におけるシュクロースの利用 H. K. Porter
9. 窒素固定（前記のものとおなじである） A. I. Virtanen
10. 生長物質とその作用
   i. オーキシン K. V. Thimann
   ii. ほかの生長物質 T. A. Bennett-Clark
   iii. 討論 K. V. Thimann
11. 植物の発育の光による統御 S. B. Hendricks
12. 成熟しつつある果実の生理 A. C. Hulme
13. Vernalization と休眠 M. Evenari
14. 生きている組織における遠距離転移 B. Huber
15. 宿主と寄生植物との生理 H. Kern
16. 植物体内における運動の力（ダーウィン100年記念シンポウジアム） E. Bünning

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1. 葉の分析と肥料の問題 W. Reuther
2. 樹木生理学の問題 P. F. Wareing

講 演

1. 原形質流動 神谷宣郎
2. 窒素代謝
   講 演
   i. 尿素およびウレイド類 K. Mothes
   ii. 遊離アミノ酸についての現在の考えかたとそれらの役わり F. C. Steward
   iii. タンパク質代謝 C. S. Hanes
3. 高等植物における個体発生の原理 M. Chailaikhjan

討 論

1. 原形質の透過性
2. 水の吸収の理論
3. みかけ上の自由空間 R. N. Robertson
4. 光合成における最近の傾向 H. Gaffron
5. 花の形成 A. Lang
6. 物質転移—理輪と証拠 S. Aronoff

第 12 部 生 態 学

A. 応用生態学，B. 個体生態学，C. 生物気象学，D. 群落生態学，E. 地図

シンポウジアム

1. 放射線生態学 John W. Wolfe
2. 微気候研究用の器具 R. Platt
3. 花粉分析のふたしかさ
4. 植物群落の数学的定義
5. 地域的規模における土地管理
6. 植生地図の特殊問題 H. Gaussen
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8. 環境ポテンシャルの指標としての植生

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以上のほか，会期前，会期中，会期後に合計 24 の見学旅行が計画されている

日本からはまだ出席者は決定していないが，学術会議で決定されれば，北村四郎，服部静夫の二人は出席できるであろう。ほかに，今井，神谷の両氏は大会組織局からの旅費の援助もあろうし，出席できるようになるとおもわれるし，なお，ほかに5～6人は日本からでる人もあろうし，アメリカ，カナダ，ヨーロッパに在留中の人で出席する人もあろうから，今度の国際植物学会には，おそらくいままでになく，おおくの日本人が出席することになろう。ついでに一言しておきたいのは，国際植物学会には——おそらくほかの学会でもそうであろうが——国の代表とか学術会議のとか，諸団体の代表とかいうかたちで出席するのでなく，それぞれひとりの植物学者として出席するのがたてまえであることは，国内の学会の大会と同様である。ただ，日本の現在の経済状態がよくないので海外旅行がつよく規制されているにすぎない。　(服部静夫記)

本 会 記 事

昭和 33 年度会計決算報告（昭和 33 年 1 月—昭和 33 年 12 月）

収入の部		支出の部	
会費	988,500	出版費	1047,788
予約購読料	455,073	発送費	106,401
一部売	22,642	編集関係費	127,738
バックナンバー売上金	40,210	図書関係費	78,740
別刷代	145,411	庶務関係費	251,612
文部省刊行補助	250,000	大会関係費	50,213
利子	25,319	支部補助	26,376
広告料	16,900	幹事手当	102,000
小計	1944,055	小計	1790,868
前年度繰越金	567,233	次年度繰越金	720,420
総計	2511,288	総計	2511,288

名 簿 訂 正

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以上の方々は先に作製いたしました名簿から落ちておりますので，ここにおわびを申しあげ，つけ加えさせていただきます。

〔削　除〕

上屋　貢　（関東）千葉県松戸市千葉大園芸学部・松戸市 3-1369

荻原玲二　（関東）横浜市港北区日赤本町 2383 公住 7-2（p. 8）

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北川昌典　（九州）大分県竹田市竹田小学校同市豊後竹田駅前岩城尾

以上の方々は名簿に二重に載っておりますので，上記の分を除きます。

Studies on Spore Germination of Hepaticae 5
Fossombronia japonica Schiffn.

by Hiroshi INOUE*

井上浩*: 苔類の胞子発芽 (5) ウロコゼニゴケ

Received December 20, 1958

The life history of the species of *Fossombronia* had been studied by Leitgeb,[1] Humphrey[2], Haupt[3], Chalaud[4], and Pande *et al*[5]. Among these authors, Leitgeb studied the sporeling of *F. pusilla* and first described the filamentous protonema for this genus. Humphrey also observed the filamentous protonema in *F. longiseta*, and Chalaud described the details of the development of *F. pussilla* and said "La phase protonemique est toujours filamenteus".

Fossombronia is a member of the Fossombroniaceae (Codoniaceae) which includes also *Petalophyllum* and *Sewardiella*[6]. This family, together with the Treubiaceae, occupy a position between the foliose and the thallose Jungermanniales. Furthermore, Mehra[7),8] recently showed that *Petalophyllum* might be thought as an ancestral form of the Marchantiales in respect of the embryology and the thallus structure. This idea may be accepted, in some degrees, from the feature of the sporeling of *Fossombronia*, phylogenetically closely related to *Petalophyllum*, whose sporeling pattern has been unknown. In this paper the author wishes to discuss this problem, describing the remarkable feature obtained from the present study.

F. japonica Schiffn. is a single representative in Japan. Formerly, in Japan, another species, *F. akiensis* Horik., was described, but it was reduced to a synonym of *F. japonica* by Hattori[9]. This species usually grows on moist sites in urban districts. The sporophytes occur in the early winter (late November to January).

The spores used in this study were collected at Nichinan of Miyazaki Prefecture on Dec. 25, 1957. They were sown about 15 days after the collection. The media for the germination were: (1) Benecke's solution, (2) Benecke's agar (2%), (3) sphagnum mats with Benecke's solution, (4) glucose solution (0.5%), (5) glucose agar (2% agar in 0.5% glucose solution), (6) sterilized soil moistened with Knop's solution. The media were infused in petri dishes, which were kept at a distance of 50 cm. from a fluorescent lamp (20 w). The experiment was carried out under the room temperature. In the case of (1) Benecke's solution the spores did not germinate.

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Germination

Spores are nearly roundish, mostly 40–47μ in diameter, with narrow wings. Spore coat has lamella (mostly reticulate). In the endospore there are numerous oil-drops and chloroplasts. The germination of the spore took place in about two weeks. The first sign of it was the swelling of spore. The size of spore became nearly two times as large as its first measurement. The spore coat ruptured irregularly and the germ-papilla appeared, which contained numerous chloroplasts and oil-drops. The oil-drops were irregular in size and stained by Sudan III. The spore coat was always attached to the basal part of the sporeling throughout the development, usually covering several cells.

The first septum was always transversal, and it separated the germ-papilla into two cells, the basal cell and a little smaller upper cell containing more chloroplasts than in the former. The basal cell did not make further cell division. The second division occurred in the upper cell with a longitudinal wall. Two cells thus formed continued further cell divisions and formed a several-celled, globose cell mass (Fig. 7, 8), whose apical cell had two cutting faces. The cells formed by this apical cell had long axes and they formed a juvenile stem of 3–4 cells thick.

When the spores were sown crowdedly in small area, the germ-papilla much elongated (Fig. 11, 12). In this case several transversal septa were formed (Fig. 13), but finally the apical cell was divided with a longitudinal wall; then it formed a several-celled, globose mass, from which a juvenile stem developed. In every case, at the apex of the juvenile stem were always differentiated the apical cells (Fig. 9, 10), which had mostly three cutting faces and produced the juvenile leaves and stem continuously.

After the differentiation of the apical meristematic cells on the juvenile stem, the juvenile leaves developed laterally (Fig. 15). The first leaf was always transversely inserted (Fig. 17), and the dorsiventrality was determined by the presence of a minute unicellular papilla on the ventral side of stem (Fig. 19, 20). On the adult plant, this papilla is not observed as mentioned in the literature. It may be noteworthy that it appears soon after the differentiation of the first juvenile leaf.

The rhizoids were formed after the differentiation of the apical meristematic cells of stem. They occurred on the ventral side of stem and their cell-wall was purplish as characteristic in the mature plant. They were always formed by the elongation of basal cells.

Usually one or two mucilage cells were observed on the margin of juvenile leaf as in the mature plant. At first, they were a little smaller than other cells in size and contained fewer chloroplasts and oil-bodies. As the development of the leaf progressed, the chloroplasts in the mucilage cell gradually disappeared and remained some oil-bodies in it. With neutral red (0.1%) the cytoplasm and the cell wall of it were stained reddish. As in *Makinoa*[10], the cytoplasm and the cell wall of the rhizoid were likewise stained with the same dye. The other cells were not stained.

Fig. 1–21. Various stages in the sporeling of *Fossombronia japonica* Schiffn. the 1, 2. Spores, ×480. 3–5. Germinations, ×480. 6–10. Globose cell masses, ×150. 11–14. Filamentous protoneme patterns, ×360. 15. Earlier stage of juvenile plant, ×150. 16. Juvenile plant, ×90. 17. First leaf, ×150. 18. Mucilage cell in first leaf (indicated with ×), ×360. 19,20. Papillae on stem, ×360. 21. Basal part of sporeling, with two rhizoids, ×150. For detailed explanation, see text,

The oil-bodies were formed firstly in the cells of the primary stem. They were very small, less than 2μ, nearly colorless, and contained more than 20 in each cell. In the protonematal cells were observed many oil-drops stained by Sudan III. However, they disappeared lately and then the oil-bodies were formed in small number per cell. They were not stained by Sudan III.

Discussion and Conclusion

As described above, there are some differences between the results obtained by the author and by Leitgeb[1] and others.[2),4)]. The difference in the protonematal form may be the most remarkable. This difference probably is thought to be caused by different light sources. As already discussed by many authors (cf. Teodoresco[11], Fulford[12,13]), the protonematal forms of hepatics are extremely affected by the environ- mental conditions.

In *F. japonica* described here it normally produces a many-celled non-filamentous protonema in the condition described firstly. However, when the spores are sown crowdedly it often produces a filamentous protonema, which agrees with that previously described by Leitgeb and others. Chalaud[4] noted that the protonema "peut être réduite à une seule cellue" in *F. pussilla*. The globose cell mass observed in this study (Fig. 6-10) can be thought to be a primitive protonema by the fact as mentioned above.

The earlier stage of the sporeling pattern of *F. japonica* and others of the genus previously described seems rather similar to that of the Marchantiales. Humphrey[2] already pointed out that the sporeling pattern of *F. longiseta* "agrees especially with that of *Fimbriaria californica*, and of *Sphaerocarpus terrestris* var. *californicus*".

The protonematal stage of *Fossombronia* is principally filamentous. In this stage it usually does not have rhizoids and has a distinct exospore attached to the basal cell. The filamentous protonema agrees with that of the *Cephalozia*-type of the foliose Jungermanniales. But two are much different in the diversion of the earlier stage of protonema caused by the envrionmental factors. In *Fossombronia* it produces normally a long germ-tube without any septum as observed in the Marchantiales, whereas in the members of the *Cephalozia*-type it does not occur. In the *Cephalozia*-type sporeling the exospore does not attach to the basal cell of the sporeling at later protonematal stage (this is significant especially for the reduced, primitive protonema of *Fossombronia*).

In the *Cephalozia*-type the differentiation of the stem from the protonema is usually direct continuation of the development from the protonema. In this point, *Fossombronia* takes different cource, and agrees with the Marchantiales, in which the protonema forms a disk that gives rise to a thallus. In *Fossombronia*, before the

* Therefore, in the *Cephalozia*-type the stage in which the juvenile plant differentiates from the protonema is, in any cases, obscure. On this, however, further discussion will be made lately.

differentiation of the stem, the apical cell of protonema forms usually a globose cell-mass, from which the stem develops*.

Among the thallose Jungermanniales, the similar patterns of sporelings may be those of *Riccardia* and *Metzgeria*, both of which form a filamentous protonema (cf. Inoue[10]). In these genera, however, the thallus is direct continuation of protonema.

From the above discussion it may be concluded that the sporeling pattern of *Fossombronia* has essential similarities to that of the Marchantiales. This conclusion may serve for Mehra's theory to some degrees.

The presence of mucilage cells on the leaf margin in adult plants of *Fossombronia* has been observed by many authors. This mucilage cells are also observed on the juvenile leaves developed at earlier stage. As shown by the vital staining with neutral red, physiological nature of the mucilage cell is differentiated from the other ordinary cells in the earlier stage, and shows similar feature to rhizoids. It may be noteworthy that the cell contains oil-bodies up to later stage, when there are observed no chloroplasts, as in the oil-cell of the Marchantiales!

Summary

The sporeling pattern of *Fossombronia japonica* Schiffn. was described and discussed with reference to that of the Marchantiales. The sporeling of *F. japonica* mostly produced a much reduced and many-celled protonema, but occasionally it produced a filamentous protonema which had several septa, as described previously in other species. The globose protonema seems to be an extreme form caused by certain environmental conditions, probably by light intensity. The sporeling pattern of *Fossombronia* much resembles that of some members of the Marchantiales in some essential features. This may serve for Mehra's theory. The mucilage cells are observed at an earlier stage and they contain oil-bodies up to the later stage.

The author is much indebted to Prof. H. Ito and Dr. S. Hattori for their many-sided advice and encouragements.

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摘　　要

本報ではウロコゼニゴケの胞子発芽の形式とともに，現在までに報告されているウロコゼニゴケ属の他の種類の発芽形式と合わせて，ゼニゴケ目の植物が行なう発芽形式との関係について論じた。ウロコゼニゴケ属では基本的には胞子が発芽すると短い糸状の原糸体をつくり，この原糸体の頂端細胞が縦分裂することによって原糸体頂端部に数細胞の集塊形成を始める（今回の研究で見られた糸状とならない原糸体は，この細胞の集塊と考えられる）。この集塊から新たに幼植物が形成される。このような発生のパターンはゼニゴケ目のそれと基本的な点で一致している。このことは最近だされた Mehra 説を裏づける一事実と考えられる。

幼植物の葉縁には mucilage cell と呼ばれる特殊な細胞が分化している（これは成熟した植物体にも見られる）。この細胞は仮根細胞と同様に neutral red で赤色に染められる。また，この細胞内では葉緑体が細胞の分化とともに次第に消失するが，油体はずっと後の時期まで変形されずに残っている。ゼニゴケ目の油細胞も発生初期にはかならず葉状体周縁部に形成され，かつ葉緑体を含むことと考え合わせて注目すべきことがらと考えられる。

Studies on the Light Controlling Flower Initiation of *Pharbitis Nil*. I. Intensity and Quality of the Light Preceding the Inductive Dark Period.

by Atsushi TAKIMOTO* and Katsuhiko IKEDA*

滝本 敦*・池田勝彦*：アサガオの花芽形成を支配する光条件について
I. 暗期前の光の強さと質

Received December 22, 1959

In many short day plants, the dark period of adequate length must be preceded by high-intensity light to initiate flower primordia, indicating that a process, the so-called high-intensity light process, plays an important role in photoperiodic induction[1),2),3),4),5)]. Liverman and Bonner[6)] reported that the requirement for this high-intensity light was replaced by feeding sugar or organic acids of the TCA cycle. Requirement of CO_2 during the high-intensity light process was also reported by Parker *et al*[7)]. The process is, therefore, regarded as the photosynthetic one producing energy sources required for the following dark process.

In preliminary experiments with *Pharbitis Nil*, in which the sugar was supplied to the plants under low intensity light followed by long dark period, no remarkable effect of the sugar was observed. The present experiments were designed to investigate whether the flower-inhibitory effect of the low intensity light preceding inductive dark period is merely attributable to the deficiency of photosynthete or to some other factors.

At the beginning, the effective intensity and the spectral sensitivity of the low intensity light for nullification of inductive effect of the following dark period was investigated. The experiments on the spectral sensitivity revealed a remarkable flower inhibitory effect of far-red light given to the plant prior to the inductive dark period. Later experiments were designed to investigate this phenomenon in detail.

Material

The material used was seedlings of *Pharbitis Nil*, strain "Violet", which will initiate floral primordia after the application of a single dark period of adequate length. Photoperiodic behaviour of this plant in the seedling stage was reported recently by Kujirai and Imamura[8)].

To obtain uniform germination, the seeds were treated with conc. H_2SO_4 for 20—30 minutes, washed thoroughly in running water for about one day, and spread on moistened sand. Two days after the treatment with H_2SO_4, germinating seeds

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were selected for uniformity and sown in 18 cm. clay pots containing garden soil, so that one pot contains 20 plants in each. They were then placed in greenhouse at 30±2°C under continuous illumination supplemented with incandescent light at night. Two days after the sowing, cotyledons expanded, and one day later the seedlings were subjected to the experimental treatment.

Methods

In the experiment in which the action spectrum of the low intensity light preceding inductive dark period for floral inhibition was studied (Exp. 3), coloured light was secured by the use of interference filter combined with coloured glasses, and 3 cm. layer of water serving as a heat filter. The light source was an incandescent lamp. Spectral energy transmittances of each filter are given in Fig. 1. In this experiment, intensity of each coloured light was adjusted to 500 erg/cm²./sec..

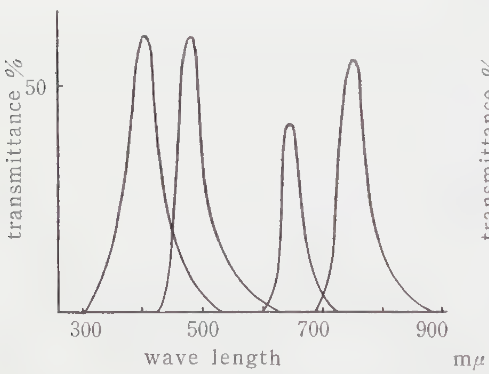

Fig. 1. Spectral energy transmittances of interference filter combined with coloured glass.

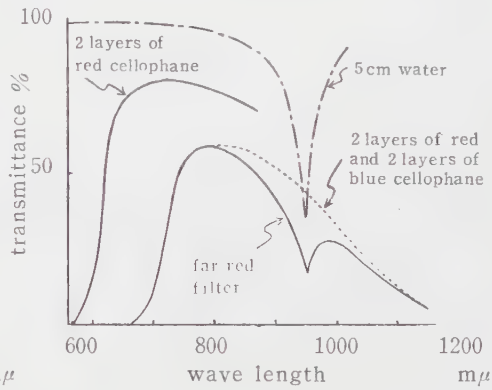

Fig. 2. Spectral energy transmittances of red and blue cellophane and water.

In other experiments, red light relatively free from far-red irradiation was secured from a pink fluorescent lamp filtered by 2 layers of red cellophane, and far-red light from an incandescent filament lamp filtered by 2 layers of red and 2 layers of blue cellophanes as described by Downs[9),10)]. Although the red cellophane filter was capable of transmitting far-red radiation, the emittance of the fluorescent lamp in the region longer than 720 mμ was negligible. The far-red light has been improved by the addition of a 5 cm. layer of water: this served to reduce the infra-red, without an appreciable decrease of the intensity of the far-red light. Spectral energy transmittances of the cellophanes and water are shown in Fig. 2.

All light measurements of the coloured light have been made with a calibrated thermopile, a Moll type, having a sensitivity of 269 mV/w/cm².. As the incandescent light comprises much heat radiation, measurement with a thermopile is inadequate, therefore, the intensity was measured with a lux meter. The intensity of daylight fluorescent light was also measured with a luxmeter, for comparision with the in-

candescent light: 1 lux of the daylight fluorescent light is equivalent to about 4.4 erg/cm.²/sec..

After the experimental treatment, plants were returned to the greenhouse, grown under continuous illumination for 2—3 weeks, and then dissected under a binocular microscope for the initiation of flower primordia. Generally, 20—40 plants were used for one lot of the treatments.

The percentage of the flowering plants, that of the plants with terminal flower and number of flower primordia per plant were used as the measures for flowering response. With some irregularities these measures run almost parallel.

Experiments were performed in an air-conditioned room of 25±1°C. Each experiment was repeated several times with almost similar results, so that only the representative results are given in the following.

Experiments and Results

Experiment 1. Plants were subjected to the incandescent light of low intensity (10 lux) for various hours preceding 16 hour dark period. Control plants were exposed to sun light preceding the 16 hour darkness. Flowering responses of the plants are shown in Table 1. The low intensity light of 8 hours preceding the inductive

Table 1. Effect of low intensity light of the incandescent lamp (10 lux) preceding 16 hour dark period upon flower initiation of *Pharbitis* seedlings.

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower primordia per plant	% of plants with terminal flower bud
$16^{h}d$	30	100	5.3	96.7
$8^{h}IL \longrightarrow 16^{h}d$	30	6.7	0.1	0
$16^{h}IL \longrightarrow$ 〃	21	0	0	0
$24^{h}IL \longrightarrow$ 〃	27	0	0	0
$32^{h}IL \longrightarrow$ 〃	29	0	0	0
$40^{h}IL \longrightarrow$ 〃	18	0	0	0
$48^{h}IL \longrightarrow$ 〃	28	0	0	0

IL: incandescent light of 10 lux
$8^{h}IL \longrightarrow 16^{h}d$: 8 hour IL is followed by 16 hour dark period.
These abbreviations will be used hereafter.

dark period reduced flower initiation heavily and only 6.7% of the plants initiated flower primordia. The low intensity light of 16 hours or more inhibited inductive effect of the following dark period completely.

Experiment 2. Plants were exposed to 8 hour incandescent light of various intensities preceding the dark period, and examined for flower inhibitory effect of the treatment. Results are shown in Table 2. Flower inhibitory effect of the incandescent light decreased with increasing intensity of the light, but even the light of 1000 lux was not strong enough to bring about a maximum flowering response.

Table 2. Effect of intensity of the incandescent light preceding 16 hour dark period upon flower initiation of *Pharbitis* seedlings. Plants were exposed to incandescent light of various intensities for 8 hours preceding the 16 hour dark period.

Intensity of incandescent light in lux	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
5	38	0	0	0
10	36	0	0	0
25	38	2.6	0	0
50	37	2.7	0	0
100	38	7.9	0.1	0
250	38	26.3	0.3	0
500	38	57.9	0.6	0
1000	38	57.9	0.7	0
Control (Sun light)	38	100	4.4	94.6

Experiment 3. To study the spectral sensitivity of the low intensity light preceding inductive dark period for flower inhibitory effect, plants were subjected to coloured light of 500 erg/cm^2./sec. for 8 hours, and subsequently given a 16 hour dark period. The coloured light used was violet (406 mμ), blue (489 mμ), red (649 mμ) and far-red (747 mμ); the spectral energy distributions of each are represented in Fig. 1.

Table 3. Effect of monochromatic light of low intensity preceding 16 hour dark period upon flower initiation of *Pharbitis* seedlings. Monochromatic light was given for 8 hours with the intensity of 500 erg/cm^2./sec.

Colour of light	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
Violet	15	87	2.1	7
Blue	13	69	1.4	0
Red	14	100	2.2	7
Far-red	13	0	0	0
White*	19	100	5.1	90

* 3500 lux from daylight fluorescent lamp and incandescent lamp.

The results represented in Table 3 indicate remarkable flower inhibitory effect of far-red light as compared with the other coloured light. Red light is most ineffective in inhibition. Experiments repeated several times gave similar results.

Experiment 4. As the remarkable flower inhibitory effect of the far-red light was ascertained in the foregoing experiment (Exp. 3), the low intensity light of daylight fluorescent lamp which comprises little far-red light was investigated for its flower inhibitory effect when followed by inductive dark period.

Plants were exposed to the daylight fluorescent light of 10 lux for 0, 8, 12, 16, 24, 30, 36, 42 and 48 hours, being arranged so as to close the treatment at the same time in all lots, and subsequently given 16 hour dark period. As the control, two lots of the plants were subjected to the daylight fluorescent light of 10 lux for 24 and 48 hours and given no dark period. Results are shown in Table 4.

Table 4. Effect of low intensity light of daylight fluorescent lamp (10 lux) followed by 16 hour dark period on flower initiation of *Pharbitis* seedlings.

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16hd	39	100	4.2	97.4
8hFL → 16hd	40	92.5	2.9	47.5
12hFL → 〃	40	87.5	2.3	5.0
18hFL → 〃	39	79.5	1.3	0
24hFL → 〃	39	97.4	2.9	20.5
30hFL → 〃	40	97.5	3.4	37.5
36hFL → 〃	38	68.4	1.2	0
42hFL → 〃	40	50.0	0.6	0
48hFL → 〃	39	79.5	1.3	0
24hFL	40	0	0	0
48hFL	40	0	0	0

FL: daylight fluorescent light of 10 lux.

Flowering responses were reduced slightly with increasing duration of the low intensity light. Even when 48 hour low intensity light of this lamp preceded the 16 hour darkness, 79.5% of the plants initiated flower primordia. Control plants which were subjected to the daylight fluorescent light of 10 lux for 24 and 48 hours initiated no flower primordia.

In this experiment, the plants exposed to the low intensity light for 12, 18, 36 and 42 hours initiated less flower buds than the other plants. They were subjected to the low intensity light either from 6 a. m. or midnight, having been exposed previously to incandescent light of about 500 lux at night. It may be supposed that the reduced flowering responses of these plants are due to this incandescent light of low intensity. But it is also conceivable that some endogenous diurnal rhythm influences the flowering responses.

Experiment 5. Flower inhibitory effect of the low intensity light preceding dark period was again investigated with respect to light sources, comparing the effect of the incandescent and the daylight fluorescent light. Results shown in Table 5 indicate clear difference in flower inhibitory effects of both lights.

Experiment 6. Low intensity light of the daylight fluorescent light (10 lux) was mixed with the far-red light of 120 erg/cm.^{2}/sec.. Plants were exposed to this light for 8, 12, 18, 24, 36, 42 and 48 hours and subsequently given a 16 hour dark period.

Table 5. Comparison between flower inhibitory effect of incandescent light and of daylight fluorescent light of 10 lux given to *Pharbitis* seedlings preceding 16 hour dark period.

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower primordia per plant	% of plants with terminal flower bud
16^hd	39	100	4.8	79.5
4^hIL ——→ 16^hd	35	80.0	1.3	0
8^hIL ——→ 〃	38	0	0	0
12^hIL ——→ 〃	37	0	0	0
18^hIL ——→ 〃	37	0	0	0
24^hIL ——→ 〃	39	0	0	0
4^hFL ——→ 16^hd	39	100	4.8	89.8
8^hFL ——→ 〃	40	32.5	0.5	0
12^hFL ——→ 〃	39	38.5	0.5	0
18^hFL ——→ 〃	40	25.0	0.3	0
24^hFL ——→ 〃	38	34.2	0.5	0

IL: incandescent light of 10 lux.
FL: daylight fluorescent light of 10 lux.

Table 6. Effect of FL+FR preceding 16 hour dark period on flower initiation of *Pharbitis* seedlings.

FL+FR: daylight fluorescent light of 10 lux mixed with far-red light of 120 erg/cm^2./sec..

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16^hd	38	100	3.2	2.6
8^hFL+FR ——→ 16^hd	39	5.1	0.1	0
12^hFL+FR ——→ 〃	38	0	0	0
18^hFL+FR ——→ 〃	39	0	0	0
24^hFL+FR ——→ 〃	39	0	0	0
30^hFL+FR ——→ 〃	40	0	0	0
36^hFL+FR ——→ 〃	31	0	0	0
42^hFL+FR ——→ 〃	38	0	0	0
48^hFL+FR ——→ 〃	38	0	0	0

The results are shown in Table 6. Eight hour application of this low intensity light reduced flowering responses strikingly and only 5.1 % of the plants initiated flower primordia. When this low intensity light period was lengthened up to 12 hours or more complete flower inhibition resulted.

The results show remarkable similarity between the incandescent light and the daylight fluorescent light mixed with the far-red light in flower inhibitory effect when given at low intensity preceding the inductive dark period.

Table 7. Reversible effect of red light and that mixed with far-red light upon flower inhibitory effect of the incandescent light of low intensity preceding the inductive dark period.
Intensity of incandescent light (IL): 10 lux.
Intensity of red light (R): 3000 erg/cm².sec.
Intensity of far-red light (FR): 5000 erg/cm².sec.

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16hd	39	100	4.6	94.9
8hIL 16hd	42	0	0	0
〃 → 1′ R → 〃	39	33.3	0.5	0
〃 → 5′ R → 〃	39	79.5	2.1	17.9
〃 → 30′ R → 〃	40	80.0	2.1	17.5
〃 → 2hR → 〃	39	82.1	2.6	25.6
〃 → 1′ R+FR → 〃	39	7.7	0.1	0
〃 → 5′ R+FR → 〃	39	10.3	0.1	0
〃 → 30′ R+FR → 〃	38	2.6	0.1	0
〃 → 2hR+FR → 〃	40	25.0	0.4	0

Experiment 7. From the above experiments, it is ascertained that flower inhibitory effect of the low intensity light of the incandescent lamp preceding dark period depends considerably upon the effect of the far-red light from the lamp. Recently, in many photomorphogenetic responses including photoperiodic response, antagonistic action between red and far-red light was observed[9),10),11),12),13),14)]. It is probable that the flower inhibitory effect of the incandescent light of low intensity is also reversed by the red light applied just before the dark treatment.

Plants were subjected to the 8 hour incandescent light of 10 lux, the plants of the first group were subsequently given red light of 3000 erg/cm.²/sce., and those of the second group the red light of the same intensity mixed with far-red light of 5000 erg/cm².sec. for 0, 1, 5, 30 minutes and 2 hours respectively, and thereafter given 16 hour dark period. Results are shown in Table 7. One minute of red light reversed flower inhibitory effect of the incandescent light to some extent, and 5—30 min. red light reversed it more effectively. The reversing effect of the red light is reduced significantly by the simultaneous application of the far-red light. Antagonistic effect of red and far-red light is observed clearly.

Discussion

Hitherto, flower inhibitory effect of low intensity light preceding the inductive dark period has been considered to be due to the lack of photosynthates as a precursor of flowering hormone or energy sources of flowering reaction[6)]. From the present investigation, it is seen that the low intensity light of the incandescent lamp has more flower inhibitory effect than that of the daylight fluorescent lamp

comprising little far-red light, and that the strong inhibitory effect of the incandescent light is to some extent attributable to its abundant far-red light and is reversed by the red light. The "red-far-red absorbing pigment system" postulated in many photomorphogenetic responses is assumed to play an important role in this case too.

Borthwick *et al.* reported for *Xanthium* that the inductive dark process is prevented by red light, and promoted by far-red light. Thus, the 30 min. far-red irradiation preceding the dark period could reduce the critical dark length by some 2 hours[12].

On the other hand, Nakayama reported that far-red light given just before the dark period inhibits flower initiation of *Pharbitis* seedlings and that the inhibition is reversed by red light[15]. The same is also the case in the present experiments. Why the far-red light preceding the dark period promotes flower initiation in *Xanthium*, and inhibits it in *Pharbitis* seedlings comes into question. To elucidate this discrepancy, more detailed studies along this line remain to be made.

Low intensity light of 8 hours or more preceding the inductive dark period has some flower inhibitory effect even if it contains little far-red light. This flower inhibitory effect of the low intensity light of long duration may be due to the deficiency of photosynthates and may correspond to that reported by Liverman *et al.*, which can be reversed by feeding sugar or organic acids.

Summary

The flower inhibitory effect of low intensity light preceding the dark period of adequate length (16 hours) was investigated in *Pharbitis* seedlings.

1) Eight hour incandescent light of 10 lux inhibits flower formation.

2) The incandescent light of 10—25 lux given for 8 hours suppresses the inductive effect of the following dark period entirely, and even that of 1000 lux is not strong enough to bring about maximum photoperiodic response.

3) Spectral sensitivity of the low intensity light for flower inhibition was studied. Far-red light has the highest inhibitory effect and red light the lowest.

4) Daylight fluorescent light of 10 lux which comprises little far-red light inhibits flowering far less than the incandescent light of the same intensity.

5) The daylight fluorescent light of 10 lux mixed with the far-red light of 120 erg/cm^2./sec. inhibits flowering remarkably when given for 8 hours.

6) Flower inhibitory effect of the incandescent light of 10 lux is reversed by red light applied just before the dark period. But the red light mixed with the far-red light has little reversing effect for the flower inhibitory effect of the incandescent light.

Flower inhibitory effect of the incandescent light is attributable to the action of the far-red light comprised in it. "Red-far-red absorbing pigment system" is supposed to play an important role in the reaction preceding inductive dark period.

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摘　　　要

アサガオ子葉に 16 時間の暗期を一度与えると花芽形成が見られるが，暗期前 10 ルックスの白熱電灯光を 8 時間与えると花芽を形成しなくなる。

1) 暗期前弱光の花芽形成抑制に対する有効波長を調べると，近赤外光がもっとも抑制効果強く，赤色光がもっとも弱い。

2) 近赤外光をほとんど含まない昼光色螢光燈光（10 ルックス）を暗期前に与えても抑制効果は非常に弱く，これに近赤外光を混ぜると抑制効果がいちじるしくなる。

3) 近赤外光の強い光を暗期前に与えると，大変いちじるしい花芽形成の抑制が見られる。

4) 10 ルックス白熱電燈光の花成抑制効果は暗期直前に 1～5 分間赤色光を与えることによって消却される。しかし赤色光と同時に近赤外光をも与えると消却効果はいちじるしく減ずる。

白熱電灯弱光の花芽形成抑制効果はそれに含まれている近赤外光の働きによるところが大きいものと考えられる。

Physiological Studies on Growth and Morphogenesis of the Isolated Plant Cell Cultured *in vitro* VI.

The Effects of Amino Acids, Adenylic Acid, Colchicine and Gibberellin

by Tadashi SANDAN*

山段　忠*：遊離植物細胞の生長成形に関する生理学的研究 VI.
アミノ酸，アデニル酸，コルヒチンおよびジベレリンの影響

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In the previous paper (Sandan, 1955)[1], the author reported that an isolated internodal cell of Characeae was able to grow and develop a new shoot and rhizoids when it was cultured in agar gel with suitable culture solutions. Furthermore, the effects of auxin, vitamins, fat solvents and other reagents upon the morphogenesis of the cell were also observed (Sandan and Ogura, 1957[2]; Sandan, 1958[3]). The present report deals with the effects of amino acids, adenylic acid, gibberellin and colchicine on the morphogenesis of the isolated internodal cell of *Nitella in vitro.*

Material and Method

In the present experiments, an isolated internodal cell of *Nitella flexilis*, which was 3.0 cm. in length and 400μ in width, was used as material. For the removal of fungi, bacteria and protozoa, the materials were kept for three or five days in a Petri dish filled with distilled water containing such antibiotics as penicillin (200 units/ml.), streptomycin (0.03 mg./ml.) and trichomycin P tablet (0.08 mg./ml.). Then the material was cultured in the vertical, normal position in a test tube filled by half with 0.6% agar gel according to the method described in the previous paper[1]. In the present work, solutions of amino acids, 3-adenylic acid, gibberellin** and colchicine in various concentrations were used as culture solution and Sörensen's phosphate buffer solution (M/100, pH 6.6) was used as basic culture solution (control) in all cases.

As to the effects induced by these reagents upon the morphogenesis of the cell, the author paid his attention to the next two points: first, the effect on the formation of a shoot and rhizoids from the cell, and second, the effect on the elongation of the shoot and rhizoids which were newly formed. The former is represented by the time required for the material to shoot and to form rhizoids after it was brought into the culture medium, and the latter is measured in terms of the length of shoot and

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** Crystalline gibberellin manufactured by the Kyôwa Fermentation Industry Co., Ltd,

rhizoid 15 days after appearance. All experiments were carried out at room temperature under diffuse light of about 80 lux.

Results

1. Effect of amino acids

All amino acids so far used, except lysine and phenylalanine, checked the morphogenesis of the cell in high concentrations and had no effect in low concentrations. But, in intermediate concentrations which were relatively low, they accelerated the morphogenesis of the cell. In the cases of lysine and phenylalanine, concentration accelerating morphogenesis was never found.

The critical concentrations of the amino acids above which the cell could not perform its morphogenetic development and the ranges of concentration in which the morphogenesis of the cell was promoted are as follows, the latter being in parentheses: arginine, 600 mg./l. (60-180 mg./l.); aspartic acid, 800 (40-160); cystine, 450 (120-220); glutamic acid, 1100 (100-160); histidine, 600 (160-280); isoleucine, 800 (120-180); leucine, 1200 (80-250); lysine, 400 (no promotion); methionine, 1000 (120-280); phenylalanine, 500 (no promotion); tyrosine, 800 (80-240)

Below these concentration ranges accelerating the morphogenesis of the materials was not different from the one in control. The results with the optimum concentration of each amino acid for morphogenesis are summarized in Table 1. According to the results, leucine, methionine, arginine, aspartic acid and glutamic acid exerted a more favorable effect on morphogenesis than the other amino acids.

Table 1. Effect of amino acids in appropriate concentrations on the morphogenesis of the cell.

L-Amino acids	Conc. mg./l	Time for shooting (days)	Length of shoot (cm.)	Time for rhizoid formation (days)	Length of rhizoid (cm.)
Control		16	1.27	15	1.58
Arginine	150	12	1.44	14	1.61
Aspartic acid	120	13	1.38	13	1.70
Cystine	180	14	1.39	14	1.64
Glutamic acid	150	12	1.42	13	1.75
Histidine	180	15	1.42	14	1.68
Isoleucine	160	14	1.37	13	1.59
Leucine	140	12	1.52	12	1.84
Lysine	120	17	1.25	16	1.54
Methionine	160	13	1.42	12	1.89
Phenylalanine	140	17	1.21	15	1.55
Tyrosine	120	16	1.29	14	1.63

2. Effect of adenylic acid

The effects of adenylic acid on the morphogenesis of the cell are shown in Table 2. As illustrated in this table, the maximum positive effect of adenylic acid was given at 5×10^{-4} M. Noticeably, adenylic acid in appropriate concentrations is favorable for shooting and rhizoid formation rather than for elongation of the shoot and rhizoids.

Table 2. Effect of adenylic acid on the morphogenesis of the cell.

Conc. (M)	Time for shooting (days)	Length of shoot (cm.)	Time for rhizoid formation (days)	Length of rhizoid (cm.)
Control	16	1.27	16	1.56
1×10^{-4}	16	1.26	16	1.57
2×10^{-4}	15	1.27	16	1.58
5×10^{-4}	12	1.28	13	1.59
1×10^{-3}	14	1.27	15	1.59
2×10^{-3}	16	1.22	17	1.53
1×10^{-2}	no shooting	——	no rhizoid formation	——

3. Effect of colchicine

The effects of colchicine upon the morphogenesis of the cell are summarized in Table 3. It is noteworthy that the elongation of both a new shoot and rhizoids was slightly promoted by application of colchicine (0.06-0.12%) but shooting and rhizoid formation were never stimulated. The concentration of colchicine most suitable for the elongation of both a shoot and rhizoids was 0.08%.

Table 3. Effect of colchicine on the morphogenesis of the cell.

Conc. (%)	Time for shooting (days)	Length of shoot (cm.)	Time for rhizoid formation (days)	Length of rhizoid (cm.)
Control	16	1.26	16	1.57
0.02	16	1.25	17	1.57
0.06	16	1.26	16	1.58
0.08	16	1.38	16	1.64
0.12	16	1.29	16	1.59
0.16	18	1.24	17	1.55
0.20	19	1.21	18	1.39
0.30	no shooting	——	no rhizoid formation	——

4. Effect of gibberellin

The effects of gibberellin upon morphogenesis of the cell are illustrated in Table 4. The most favorable concentration of gibberellin for the morphogenesis was 14 mg./l.. Judging from the results, gibberellin seems to be favorable for the elongation of a new shoot.

Table 4. Effect of gibberellin on the morphogenesis of the cell.

Conc. (mg./l.)	Time for shooting (days)	Length of shoot (cm.)	Time for rhizoid formation (days)	Length of rhizoid (cm.)
Control	16	1.25	16	1.56
2	16	1.25	16	1.55
5	16	1.24	16	1.57
10	16	1.25	16	1.56
12	15	1.39	16	1.57
14	14	1.43	15	1.58
16	15	1.28	15	1.57
18	15	1.25	16	1.57
20	16	1.24	17	1.54
30	23	0.94	26	1.12
40	no shooting	——	no rhizoid formation	——

Discussion

Colchicine arrests the mitotic process chiefly in the metaphase by causing failure of the mitotic spindle to form and function (Ludford, 1936)[4]. Kato (1955)[5] described that the *Equisetum* spore cultured on a medium containing a large quantity of colchicine develops into a giant globe of about 10 to 15 times its normal volume, cell division being perfectly inhibited, and that cell-differentiation does not eventually occur. And he postulated that colchicine acts not only as a polyploid inducer, but also as an effective modifier of rhizoid-differentiation and morphogenesis. But, later, he (1957)[6] observed in *Dryopteris* spore that the rhizoids are always capable of differentiating, even in higher concentrations of colchicine, and the rhizoidal protonema occurs in a high frequency. In the present work using *Nitella* cell, which is a coenocyte, colchicine in appropriate concentrations exerted no effect on shooting and rhizoid formation but it slightly promoted the elongation of both a new shoot and rhizoids.

Kato (1957)[7] tested sodium adenosine triphosphate and adenylic acid in order to induce the division of rhizoidal nucleus of *Dryopteris*. He frequently observed amitotic figures and dumbbell-shaped nuclei there but he never found a rhizoid having two

nuclei. In the present experiment, shooting and rhizoid formation in the isolated *Nitella* cell were certainly accelerated by application of adenylic acid in adequate concentrations. From the important rôles of adenylic acid as a constituent of nucleic acids or other biochemical functions of it thus far known, it seems plausible that adenylic acid promoted the morphogenesis in the cell of *Nitella*.

As reviewed by Stowe and Yamaki (1957)[8], there are many reports in regard to the action of gibberellin. Kuraishi and Hashimoto (1957)[9] reported that the expansion of the first leaf of seedlings of *Raphanus* increased up to 25% by application of gibberellin (10 mg./1.). Kato (1955)[10] found that gibberellin had no effect on roots of onions but it strongly promoted the growth of rhizoid cells of ferns. Sachs and Lang (1957)[11] observed that the cell division of shoot apex in *Hyoscyamus* was accelerated by the treatment with 25 mg./1. of gibberellin. In the present experiments, the morphogenesis in the cell of *Nitella*, especially the elongation of a new shoot, was promoted by application of gibberellin in low concentrations as illustrated in Table 4.

Sawada (1958)[12] found the following phenomena: The pollen grain of *Paris* does not germinate on the sugar agar medium, but adding aspartic acid, glutamic acid, histidine or cysteine to the culture medium, the pollen grain is enabled to germinate readily. The germination of the pollen grain of *Oryza* was markedly stimulated when arginine, valine or alanine was added to the medium.

As shown in Table 1, the morphogesis of the cell of *Nitella* was certainly accelerated by application of most amino acids so far used. And above all, leucine, methionine, glutamic acid, aspartic acid and arginine were favorable for the morphogenesis of the cell.

Summary

The morphogenesis of the isolated internodal cell of *Nitella flexilis* cultured *in vitro* was promoted by application of adenylic acid, gibberellin and most amino acids, e. g., leucine, methionine, glutamic acid, aspartic acid and arginine in appropriate concentrations. Amino acids had a positive effect on the formation and the elongation of both the shoot and rhizoid. Adenylic acid was favorable for shoot and rhizoid formation. Gibberellin was suitable for the elongation of the shoot.

Colchicine exerted no effect on formation of shoot and rhizoid at whatever concentrations, but it could accelerate the elongation of both the shoot and rhizoid already formed.

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摘　　要

フラスモの遊離節間細胞の生長•成形は，適当濃度のアデニル酸やジベレリンあるいはロイシン，メチオニン，アスパラギン酸，グルタミン酸，アルギニンなどのアミノ酸をそれぞれ加えることによって促進される。その場合アデニル酸は細胞よりの出芽•出根に，ジベレリンは新生した芽の伸長に，またアミノ酸は芽と仮根の新生とその伸長に強い効果を及ぼす。

コルヒチンはある濃度範囲で新生した芽と仮根の伸長生長をわずかに促がすことができるが，出芽•出根に対する促進効果はみとめられない。

A Sensitized *Avena* Curvature Test and Identification of the Diffusible Auxin in *Avena* Coleoptile

by Hiroh SHIBAOKA* and Toshio YAMAKI*

柴岡弘郎*・八巻敏雄*　アベナ屈曲試験の一増感法と，拡散オーキシン同定へのその応用

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Introduction

Studies concerning auxins existing in plant extract have been made by many workers, and the existence of several growth substances, not yet identified, has been reported[1),2)]. However, the chemical nature of auxin diffused from plant tissues into agar blocks has been investigated by very few, though such diffused auxin has been regarded as the substance having close connection with phototropic or geotropic movement of intact plant.

As the reason why the study of such an important substance that may control these naturally occurring phenomena of intact plant has not yet been made, it is possible to mention that the quantity of diffusible auxin is too small to be detected, after several times of various fractionations.

To make the study easier, it may be useful to find out a more sensitive method of detection for small quantity of auxin.

Recently, some heavy metal ions, such as Co^{++}, Mn^{++}, Fe^{++}, Ni^{++}, have been found to promote the growth of *Avena* coleoptile sections and pea epicotyl sections under natural conditions and in the case of growth induced by addition of auxin[3),4)]. And the authors attempted to increase the sensitivity of *Avena* curvature test, using these heavy metal ions.

The present paper reports the promotive effect of Fe^{++} on *Avena* curvature test and gives an example of our experiments which have been carried out using the new sensitive *Avena* curvature test, i. e. the chromatographical study of the nature of an auxin obtained by diffusion into small agar blocks form *Avena* coleoptile tips.

Studies on the mechanism of the promoting action of ferrous ion will be reported in another paper[5)].

Experiments and Results

(1) The effect of $FeSO_4$ on *Avena* curvature test.

Victory oat No. 1 was used as a test plant. Seedlings were grown on sand for *Avena* curvature test, the method described by Thimann[6)] was used. Agar blocks

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used for the test were 2 % pure agar blocks ($2\times2\times2$ mm^3.) and 12 pieces of these were soaked for 3 hours in 20 ml. of a test solution. Test solutions were adjusted to pH 5.0 with diluted H_2SO_4, since the addition of $FeSO_4$ could change the pH of the solution. Curvatures of *Avena* coleoptiles were measured 90 minutes after the application of agar blocks.

Fig. 1 represents the results of an experiment using test solutions containing both 0.025 mg./l. of IAA and $FeSO_4$ at various concentrations. The addition of $FeSO_4$ to the test solution increases obviously the IAA induced curvature of *Avena* coleoptile. The optimum concentration of Fe^{++} is around 3×10^{-3} to 10^{-2}M, and Fe^{++} in these concentrations increases the curvature about 200 %. In case of Fig. 2, $FeSO_4$ was used at its optimum concentration, i. e. 5×10^{-3}M, and the IAA concentration was varied. Ferrous ion in its optimum concentration increases the curvature by about 200, 110, 33 % in presence of IAA in concentration of 0.025, 0.050, 0.100 mg./l., respectively. As far as our experiments are concerned, the lower the concentration of IAA, the more obvious the promotion due to ferrous ion seems to be. In order to ascertain whether or not ferrous ion increases the curvature induced by auxin other than IAA, the effect of $FeSO_4$ on curvature of *Avena* coleoptile induced by NAA was exmined. Table 1 is the result of this experiment. This result shows that ferrous ion promotes not only the coleoptile curvature induced by IAA but also that induced by NAA.

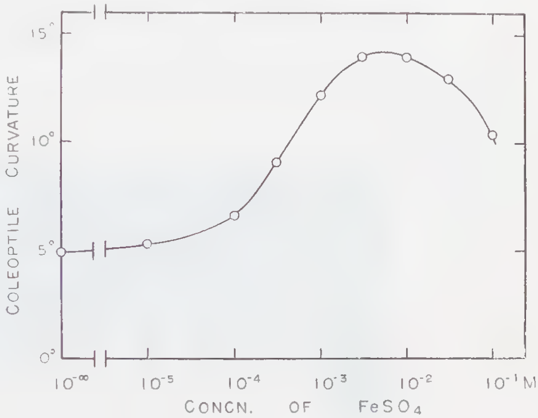


Fig. 1. Effect of $FeSO_4$ on the *Avena* coleoptile curvature induced by IAA. IAA was used at the concentration of 0.025 mg./l. Curvature was measured 90 minutes after the application of the agar block.

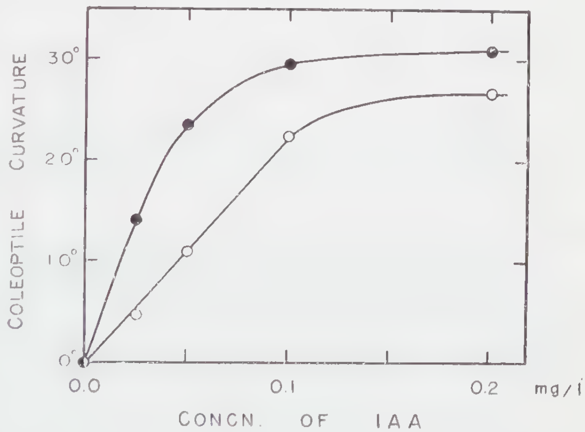


Fig. 2. Effect of $FeSO_4$ on the *Avena* coleoptile curvature induced by IAA. $FeSO_4$ was used at the concentration of 5×10^{-3}M. Curvature was measured 90 minutes after the application of the agar block.

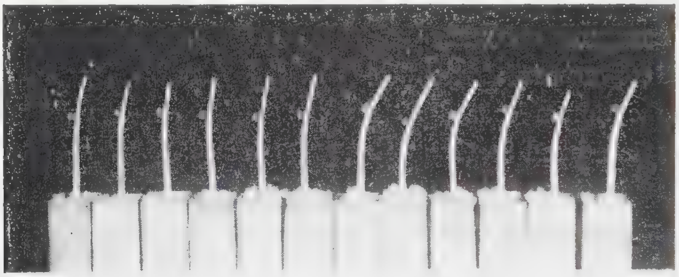
—○—: IAA alone —●—: IAA+$FeSO_4$

The above described results show that the application of $FeSO_4$ to *Avena* curvature test is very effective to detect a small quantity of auxin, especially that ferrous

Table 1

Effect of $FeSO_4$ on the *Avena* coleoptile curvature induced by NAA. NAA was used at the concentration of 0.3 mg./1. and $FeSO_4$ was used at 5×10^{-3}M. Curvature was measured 90 minutes after the application of the agar block.

	Curvature in degrees
NAA	7.3
NAA + $FeSO_4$	16.0



IAA 0.025 mg./1. alone

IAA 0.025 mg./1. $FeSO_4$ 5×10^{-3} M

Fig. 3. Effect of $FeSO_4$ on the *Avena* coleoptile curvature induced by IAA.

ion in its concentration at 5×10^{-3}M increases the sensitivity of *Avena* curvature test by about 3 times.

(2) Identification of the auxin diffused from *Avena* coleoptile tips into agar blocks.

Using the above described sensitive *Avena* curvature test, the nature of the diffusible auxin, obtained from *Avena* coleoptile tips, was identified by paper chromatography.

When the height of *Avena* seedling grown in the dark room reached about 2.5 cm., a coleoptile tip 3 mm. in length was harvested, and placed on a pure agar block (2%, $2\times2\times2$ mm^3.) and kept in darkness at 25° for 3 hours. By this procedure, about 600 agar blocks containing the diffusible substances were obtained from the same number of coleoptile tips. These agar blocks, about 5 ml. in total volume, were soaked into 25 ml. of cold ether and kept at 0° for 12 hours. The ether extract was removed and the agar blocks were washed twice with 10 ml. of fresh ether, and the ether extracts were gathered and evaporated to dryness. This will be called the ether fraction. The residue of the ether extraction, the remaining agar blocks, was soaked in 40 ml. of cold water and kept at 0° for 12 hours. The water extract was removed and the agar blocks were washed twice with 10 ml. of fresh water. These water extracts were gathered and evaporated under reduced pressure at 50°. This will be named the water fraction. Each fraction was dissolved in 2 ml. of water and divided into two equal parts. One ml. of water was added to one part of

each fraction and the same volume of 10^{-2}M $FeSO_4$ solution was added to the other part. In this way, four kinds of test solutions were prepared, i. e. (1) ether fraction+water, (2) ether fraction+$FeSO_4$, (3) water fraction+water and (4) water fraction+$FeSO_4$. Twelve pieces of agar blocks were soaked in each test solution for 3 hours, and were used for *Avena* curvature test. The auxin activity which was found in each test solution was shown in Table 2.

Table 2

Auxin activities of the two fractions of the diffused substances obtained from *Avena* coleoptile tips, and the effect of $FeSO_4$ on the *Avena* coleoptile curvature induced by the diffusible auxin. The auxin activity was expressed by the degree of *Avena* coleoptile curvature measured 90 minutes after the application of the agar block. $FeSO_4$ was used at the concentration of 5×10^{-3}M.

	Auxin activity
Water fraction + Water	0
Water fraction + $FeSO_4$	0
Ether fraction + Water	3.85
Ether fraction + $FeSO_4$	8.64

The two water fractions showed no activity by the *Avena* curvature test, this fact indicates that all the active substances in agar blocks were ether soluble. Moreover, the water fraction did not induce any curvature even 180 minutes after the beginning of the test. Among the two ether fractions, the one which has ferrous sulfate in it promotes the curvature of *Avena* coleoptile more than the one having no ferrous sulfate. This phenomenon indicates that Fe^{++} promotes the curvature of *Avena* coleoptile, which is induced by the auxin obtained from *Avena* coleoptile tips by diffusion.

As the ether fraction showed the auxin activity, the chromatographical analysis of this fraction was performed. Toyo No. 51 filter paper strips, 3 cm. in width, were used and the ascending method was applied. As developing solvent systems, the following mixtures were used: *iso*-propanol-water-ammonia (10:1:1, v/v), ethanol-water (7:3, v/v) and *n*-butanol saturated with water-acetic acid (200:1, v/v). Solvents were allowed to run 19.5 cm. up from the starting line and the strips were dried. The dried chromatogram, having 20 cm. in length from 5 mm. below the starting line up to the solvent front, was cut into 10 equal pieces and each division was dipped into 0.2 ml. of 5×10^{-3}M $FeSO_4$ solution. Every group of 12 pieces of 2% agar block ($2\times2\times2$ mm^3.) was placed on each of the 10 pieces of the above mentioned wetted filter papers for 3 hours and these agar blocks were used for *Avena* curvature test. Results are summarized in Fig. 4. In every case, only one region having auxin activity was found on a chromatogram, and R_f value of this region always coincided with that of IAA in control strip. The experiment using ammoniacal *iso*-

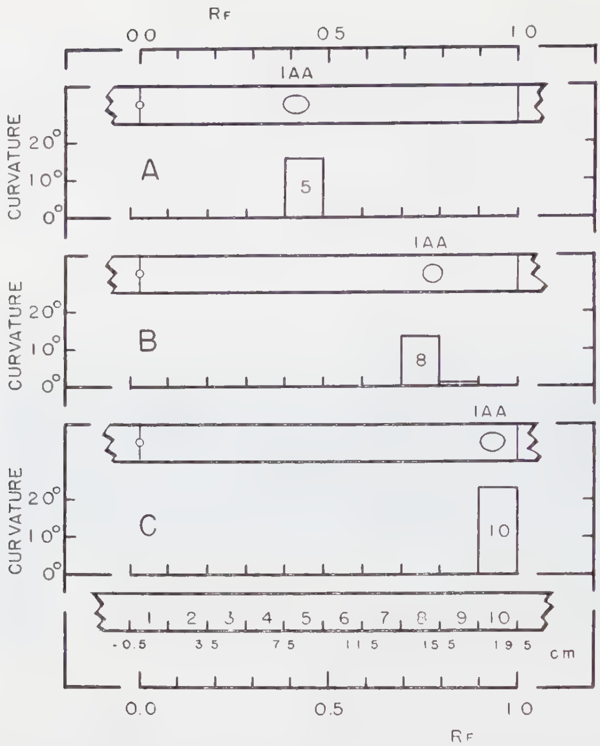


Fig. 4. Three chromatograms of diffusible auxin in *Avena* coleoptile tips, developed by the following solvent mixtures:

A: *iso*-propanol-water-ammonia (10:1:1, v/v)
B: ethanol-water (7:3, v/v)
C: *n*-butanol sat. with water-acetic acid (200:1, v/v).

Control strips were colored with Salkowski-Tang's reagent. Auxin activity was measured by the sensitized *Avena* curvature test and expressed as the degree of the coleoptile curvature. The lowest paper strip indicates the distance of each zone (in cm.) from the starting line.

propanol system was repeated four times, and the same results were obtained, i. e. auxin activity did not appear except in No. 5 zone of the chromatogram as indicated in Fig. 4. Furthermore, other zones than No. 5 showed no activity even in prolonged *Avena* curvature test, in which the measurement of the curvature was made 180 minutes after the application of the agar block.

Discussion

Based on the rigid selective permeability of cut surface of coleoptile, it has been supposed that the *Avena* curvature test is very little affected by the presence of a salt. In fact, Cu^{++}, Mn^{++} and Ni^{++} at the concentration of 10^{-3}M, have been found to have no effect on this test[7]. Moreover, at the same concentration, even KCN does not show any effect (unpublished data of the authors). The present investigation demostrates, however, the promotive effect of ferrous ion on *Avena* curvature test, even at the concentration of 10^{-4}M. This result probably requires further in-

vestigations concerning the permeability of the cut surface to the various salts. And the question why the auxin induced curvature of *Avena* coleoptile is promoted by ferrous ion, seems to be an interesting and important problem. Further considerations about this point will be given in another paper[5].

When the concentration of auxin is rather low, ferrous ion, in its optimal concentrations, promotes the sensitivity of *Avena* curvature test about three times. This will certainly contribute to the detection of small quantities of auxin, especially to the study of diffusible auxin, because such a study depends so much on the accuracy and sensitivity of the test method.

The investigations of the auxins in *Avena* coleoptile tips have been made by many workers[8),9),10),11),12]. Wildman *et al*[12], demonstrated that the substance extracted form *Avena* coleoptile tips gave pink color with Salkowski's reagent, and the quantity of IAA estimated by the intensity of color reaction coincided fairly well with that obtained by bioassay.

Raadts *et al*[9] obtained two growth substances from ground tips by water extraction. They reported that one of them seemed to be IAA and the other, not yet be identified, was probably an unstable substance, having high molecular weight and low "Wanderungsfähigkeit". Here, the authors made the study of the auxin diffused from *Avena* coleoptile tips into agar blocks using the above mentioned sensitive *Avena* curvature test and they demonstrated chromatographically that no other growth substance was transporated into agar blocks than IAA.

Therefore, the "2 Wuchsstoff" of Raadts and Söding is probably a substance having low "Wanderungsfähigkeit" as they reported. According to the nature of the "2 Wuchsstoff", this substance seems to have no direct relation with the growth and the growth movement of plant. And it has been reported also that the degree of phototropic or geotropic movement of *Avena* coleoptile depends very closely on the quantity of the auxin transported from coleoptile tip[7]. And the present paper demonstrates that the diffusible auxin of *Avena* coleoptile is only IAA. So, it may be possible to say that the auxin having close connection with the growth movement is only IAA, at least in the case of *Avena* coleoptile.

In various plant materials, the existence of several growth substances has been demonstrated[1),2]. To determine which is the diffusible auxin of such plant, may be a very interesting and important work, because such work probably will give us a way to make clear which is the auxin having direct connection with the growth of plant.

Summary

(1) Curvature of *Avena* coleoptile induced by IAA is increased in presence of ferrous sulfate. The optimum concentration of ferrous sulfate for the promotion of *Avena* coleoptile curvature is about 5×10^{-3}M.

(2) And in this concentration, ferrous sulfate increases the sensitivity of *Avena* curvature test about three times.

(3) Curvature of *Avena* coleoptile induced by NAA is also increased in presence of ferrous sulfate.

(4) The auxin diffused from *Avena* coleoptile tips into agar blocks is identified as IAA according to the above mentioned sensitive *Avena* curvature test. And no growth substance other than IAA was found in agar blocks.

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摘　　要

植物組織より寒天片中に拡散されるオーキシンは，生長運動などと密接な関係をもっていると考えられる。しかしそのオーキシン量がわずかなためこれについての研究はあまりなされていない。このような研究を容易にする方法の一つとしてオーキシンの検出法をより感度の高いものにすることが必要と考えられる。その試みとしてアベナ幼葉鞘切片，エンドウのエピコチル切片の生長に対し，またこれらにおよぼす生長素の作用に対し，促進的に働らくことが知られている金属イオンを応用して見た。$FeSO_4$ は 0.025 mg./l. の IAA によっておこるアベナの屈曲に対し，濃度 3×10^{-7}～10^{-2}M で約 200％（最大）の促進作用を示す。また IAA を 0.025, 0.050, 0.100 mg./l. とすると，5×10^{-3} M の $FeSO_4$ はそれぞれ約 200, 100, 33％の促進を示す。同じような $FeSO_4$ の促進作用は NAA によっておこる屈曲についても見られた。したがって 5×10^{-3}M の $FeSO_4$ を用いることにより，屈曲試験による生長素検出の感度を高めることができる。

そこでこの感度を高めた屈曲試験を使用しアベナ幼葉鞘先端より寒天片中に拡散される生長素の同定をペーパークロマトグラフィーを利用して行なった。約 600 本の幼葉鞘先端を 2％ の寒天片上に立て，3 時間拡散を行なわせた。まず拡散された物質のうちアベナ屈曲試験に働らきを示すものがすべてエーテル可溶であり，またこの物質による屈曲も $FeSO_4$ により増大されることをたしかめた。つぎにこのエーテル可溶な部分をエタノール－水（7:3, v/v），イソプロパノール－水－アンモニア（10:1:1, v/v），水飽和 *n*-ブタノール－酢酸(200:1, v/v) を用いて展開し，3 種類のクロマトグラムを作った。その結果 $FeSO_4$ を用いたアベナ屈曲試験に働らきを示す場所はどの場合でも，対照とした濾紙上の IAA の場所と同じであった。アベナ幼葉鞘先端には IAA 以外の生長物質も存在しているが (Raadts 等, 1957)，上に述べたわたくしたちの実験結果は幼葉鞘の生長運動などに直接関係している物質は IAA だけであることを示している。

On the Chlorophylls in Some Angiospermous Seeds

by Kiyonobu TOYODA*

豊田清修*: 被子植物種子のクロロフィルについて

Received January 12, 1959

Generally chlorophylls are not present in the seed or seedling grown in the dark. Concerning chlorophyll formation in the dark, however, various works have been reported. Myers[1] found chloropoylls produced by *Protococcus* sp. and *Chlorella vulgaris* in the dark and noticed that no significant difference in quantity has been found between the chlorophylls produced in the light and in the dark. Schou[2] studied the formation of chlorophyll in excised embryos of *Pinus Jeffreyi* and found that the presence of glucose or sucrose in the medium on which the embryos were grown caused a slight formation of chlorophyll. According to his description, Smith and Koski (1948) found that cotyledons of *P. Jeffreyi* which germinated in the dark contained chlorophyll *a* and *b* in the usual ratio. Bogarad[3] studied the factors associated with synthesis of chlorophyll in the dark in seedling of *P. Jeffreyi*. Koski *et al.*[4] informed that the action spectrum for the transformation of protochlorophyll to chlorophyll *a* in normal and albino corn seedling and the position of the maxima in the action spectra are in agreement with the protochlorophyll extracted. Pearson *et al.*[5] detected chlorophylls in the green pigments of aspen bark by using spectrophotometer and found the chlorophyll is photosynthetically active.

Regarding the seed, Wagenknecht *et al.*[6] informed that the loss of chlorophylls in raw green peas by storage in frozen state at 0° F for 1 and 5 years was found, while blanched peas of the same lots stored under identical conditions showed no such a loss. Some seeds have green pigments in the inner part of them, and yet these have not been investigated satisfactorily.

The present paper is concerned with the chlorophylls in some angiosperms, such as Indian lotus, pea, soy bean, orange, kumquat and pumpkin. The pigments in these seeds were detected by paper chromatographic analysis and some of those ascertained by the absorption spectra.

Just before submitting this paper to the editor, the writer had an opportunity to read a paper of Hejnowicz[13], in which he reported the presence of proto-

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chlorophyll *a* in root tips of wheat seedlings and other 15 species of pteridophytes, gymnosperms and angiosperms grown in the dark. In this paper he quoted a work of Burström and Hejnowicz[12], in which it was indicated that chlorophyll occurred in the innermost layer of the cortex of wheat roots, the growth of which had been inhibited by 1-naphthyl-acetic acid. The writer wonders if these very interesting facts have something to do with chlorophyll formation in seeds.

Materials

1. *Nelumbo nucifera* Gaertn. (Indian lotus)

The seed of this species is covered by hard pericarp and the embryo has two white cotyledons and an apparently green plumule which has two deep green juvenile leaves being responsible for the color. The green plumules of ripe seeds as well as those in various stages of ripening* were used.

2. *Pisum sativum* L.

The color of green pea is due to the cotyledons and observable through the white semitransparent seed-coat. The color is not equally distributed in the whole cotyledons, and only the external green tissue was therefore used in the present study.

3. *Glycine Max* Merrill (soy bean)

Among many forms of soy bean, there are found some which have green cotyledons. Also in this case the green color is distributed only in external tissue and internal part is colored generally brown; so the green part was exclusively used for the experiment.

4. *Citrus Junos* Tanaka, *C. Tamurana* Hort. ex Tanaka, *C. depressa* Hayata, *C. Oto* Hort. ex Y. Tanaka[8], *C. Keraji* var. *Kabuchii Y. Tanaka*[8], *C. sinensis* Osbeck, *C. nobilis* Lour. and *C. Tachibana* Tanaka

In *Citrus* seeds, the polyembryony is occasionally observed, and each embryo is either white or green in color. The green embryos were used in the present work.

5. *Fortunella margarita* Swingle

Seeds of genus *Fortunella* have green cotyledons in general and those of *F. margarita* were used.

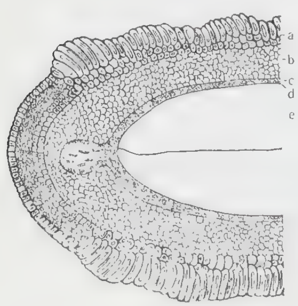

Fig. 1. Part of a cross section of a seed of *Cucurbita maxima* Duch. ×13 a: Sclerenchyma b: White parenchyma c: Green parenchyma d: Endopleura e: Cotyledon

6. *Cucurbita moschata* Duch., *C. maxima* Duch.

In the seeds of these species there are, as is well known, green pimgents. Anatomically the following several layers of tissue were found in the seed of *C. maxima*; outer tissue consists of the sclerenchyma(a) with several layers of various-sized cells, and inner tissue consists of white parenchyma(b) of several layers, and inside the layer there is a green, thin tissue of parenchyma(c). Inside this green tissue is found an endopleura(d) which wraps the embryo having two white cotyledons(e) (Fig. 1). The green

* The writer[9] divided the process of fruit formation of *Nelumbo nucifera* into seven stages.

tissue near the part of funicle is thicker than the other green part of the parenchyma and this was employed in the present study.

Methods

At first the green pigments were analyzed by paper chromatographical method. Various methods of paper chromatography of chlorophylls have been developed, but being most simple and suitable, the method described by Asami [10] was used for the most part. As the solvent, methanol-acetone mixture (1:1) was used. About 5 ml. of the solvent was added to 0.1–0.2 g. samples, which were put in the dark several hours or a day, and the extracted pigments were examined by paper chromatography. While the green pigments are being extracted from the seeds, it is exclusively necessary to avoid the light, because Koski[11] reported that in the dark-grown seedlings of *Zea mays*, protochlorophyll transformed rapidly into chlorophyll *a* on intense illumination. Therefore the experiments were performed under the weak, indirect light as quickly as possible. As the developing agents, benzene, carbon tetrachloride, toluene and xylene were used. Paper strips (size 2×20 cm.) were made from a sheet of No. 50 or 53 Toyo filter paper, and paper chromatograms were run by the ascending method. Absorption curves of the green pigments were taken with the aid of a Beckman Recording Spectrophotometer Model DK-2.

Results

The results of the paper chromatography of the pigments in the seeds are shown in Table 1.

Table 1. Results of paper chromatography on the chlorophyll pigments in some seeds (at 16–25°).

Solvent	Rf Value and Color			
	(1) Plumule of *Nelumbo nucifera*			
	(a) Plumule in normal seeds			
B*	0.32(YG)**	0.43(BG)	0.97(Y)	
C	0.23(YG)	0.32(BG)	0.57(Y)	0.85(Y)
T	0.19(YG)	0.22(BG)	0.29(Y)	0.82(Y)
X	0.29(YG)	0.35(BG)	0.40(Y)	0.72(Y)
	(b) Plumule in unripe seeds (third stage)			
B	0.26(YG)	0.95(Y)		
C	0.21(YG)	0.31(Y)	0.83(Y)	
T	0.20(YG)	0.90(Y)		
X	0.19(YG)	0.22(Y)	0.89(Y)	

(c) do. (fifth stage)

B	0.32(YG)	0.35(BG)	0.80(Y)		
C	0.19(YG)	0.26(BG)	0.51(Y)	0.64(Y)	
T	0.24(YG)	0.30(BG)	0.40(Y)	0.84(Y)	
X	0.20(YG)	0.27(BG)	0.31(Y)	0.82(Y)	
	(2) Cotyledon of *Pisum sativum*				
B	0.24(YG)	0.31(BG)	0.85(Y)		
C	0.33(YG)	0.42(BG)	0.70(Y)		
T	0.21(YG)	0.27(BG)	0.74(Y)		
X	0.24(YG)	0.30(BG)	0.82(Y)		
	(3) Cotyledon of *Glycine Max*				
B	0.35(YG)	0.56(Y)			
C	0.29(YG)	0.52(Y)			
T	0.35(YG)	0.52(Y)			
X	0.28(YG)	0.50(Y)			
	(4) Embryos of *Citrus* spp.				
	(a) *C. Junos*				
B	0.28(YG)	0.89(Y)			
C	0.25(YG)	0.90(Y)			
T	0.18(YG)	0.90(Y)			
X	0.21(YG)	0.85(Y)			
	(b) *C. Tamurana*				
B	0.31(YG)	0.78(Y)			
C	0.33(YG)	0.83(Y)			
T	0.22(YG)	0.67(Y)			
X	0.19(YG)	0.68(Y)			
	(c) *C. depressa*				
C	0.19(YG)	0.32(BG)	0.70(Y)	0.80(Y)	
	(d) *C. Keraji* var. *Kabuchii*				
C	0.26(YG)	0.33(BG)	0.60(Y)	0.64(OY)	0.99(Y)
T	0.32(YG)	0.58(BG)	0.92(Y)		
X	0.34(YG)	0.57(BG)	0.83(Y)		
	(e) *C. Oto*				
B	0.31(YG)	0.94(Y)			
C	0.33(YG)	0.72(Y)			
T	0.18(YG)	0.78(Y)			
X	0.20(YG)	0.73(Y)			

(f) *C. sinensis*

B	0.36(YG)	0.92(Y)		
C	0.31(YG)	0.85(Y)		
T	0.33(YG)	0.82(Y)		
X	0.32(YG)	0.78(Y)		

(g) *C. nobilis*

B	0.27(YG)	0.50(YG)	0.82(Y)	
C	0.36(YG)	0.57(Y)		
T	0.26(YG)	0.35(YG)		
X	0.28(YG)	0.35(YG)		

(h) *C. Tachibana*

B	0.30(YG)	0.33(BG)	0.38(Y)	0.85(Y)
C	0.38(YG)	0.38(BG)	0.50(Y)	0.70(Y)
T	0.22(YG)	0.25(BG)	0.27(Y)	0.75(Y)
X	0.24(YG)	0.28(BG)	0.33(Y)	0.75(Y)

(5) *Fortunella margarita*

B	0.35(G)	0.70(Y)	0.85(Y)	
C	0.35(YG)	0.44(BG)	0.55(Y)	0.65(OY)
T	0.22(YG)	0.25(BG)	0.52(Y)	0.75(Y)
X	0.25(YG)	0.28(BG)	0.53(Y)	0.74(Y)

(6) Green pigments in the seed-coat of *Cucurbita* spp.

(a) *C. moschata*

B	0.22(YG)	0.98(Y)	
C	0.31(YG)	0.50(Y)	0.99(Y)
T	0.19(YG)	0.68(Y)	0.82(Y)
X	0.18(YG)	0.70(Y)	0.99(Y)

(b) *C. maxima*

B	0.16(YG)	0.90(Y)	0.99(Y)
C	0.13(YG)	0.85(Y)	0.99(Y)
T	0.15(YG)	0.80(Y)	0.99(Y)
X	0.18(YG)	0.75(Y)	0.99(Y)

(c) do. (the seed-coat being uncovered, the inner part of the same seed was exposed to the sunlight a day and examined on the next day)

B	0.21(YG)	0.95(Y)
C	0.17(YG)	0.55(Y)
T	0.18(YG)	0.80(Y)
X	0.17(YG)	0.73(Y)

* B: benzene, C: CCl_4, T: toluene, X: xylene

** YG: yellowish green, BG: bluish green, Y: yellow, OY: orange yellow

Table 2. Results of paper chromatography of chlorophylls in some angiospermous leaves (at 16°-25°).

Solvent	Rf Value and Color				
	(1) Leaf of *Nelumbo nucifera*				
	(a) Leaf which germinated from the seed in the dark				
B	0.18(YG)	0.23(BG)	0.92(Y)		
C	0.17(YG)	0.29(BG)	0.86(Y)		
T	0.12(YG)	0.17(BG)	0.79(Y)		
X	0.12(YG)	0.15(BG)	0.79(Y)		
	(b) Leaf which germinated from the seed in the light				
B	0.20(YG)	0.26(BG)	0.79(Y)		
C	0.15(YG)	0.20(BG)	0.50(Y)	0.70(Y)	
T	0.14(YG)	0.19(BG)	0.56(Y)	0.78(Y)	
X	0.11(YG)	0.18(BG)	0.50(Y)	0.73(Y)	
	(c) Floating leaf on the surface of the water				
B	0.24(YG)	0.29(BG)	0.90(Y)	0.99(Y)	
C	0.10(YG)	0.26(BG)	0.60(Y)	0.99(Y)	
T	0.24(YG)	0.32(BG)	0.85(Y)	0.98(Y)	
X	0.18(YG)	0.24(BG)	0.76(Y)	0.98(Y)	
	(d) Normal leaf				
B	0.22(YG)	0.32(BG)	0.92(Y)	0.98(Y)	
C	0.15(YG)	0.23(BG)	0.51(Y)	0.60(Y)	0.97(Y)
T	0.17(YG)	0.26(BG)	0.58(Y)	0.83(Y)	0.97(Y)
Y	0.17(YG)	0.23(BG)	0.52(Y)	0.80(Y)	0.97(Y)
	(2) Leaf of *Dolichos Lablab*				
	(a) Albino leaf				
B	0.25(Y)	0.65(Y)	0.97(Y)		
C	0.14(Y)	0.22(Y)	0.52(Y)		
T	0.21(Y)	0.57(Y)	0.81(Y)		
X	0.18(Y)	0.42(Y)	0.71(Y)		
	(b) Leaf which germinated in the dark and grown up in the light				
B	0.15(YG)	0.21(BG)	0.28(Y)	0.74(Y)	0.92(Y)
C	0.15(YG)	0.19(BG)	0.22(Y)	0.64(Y)	0.99(Y)
T	0.10(YG)	0.16(BG)	0.19(Y)	0.60(Y)	0.82(Y)
X	0.10(YG)	0.13(BG)	0.19(Y)	0.49(Y)	0.73(Y)
	(c) Normal leaf				
B	0.16(YG)	0.20(BG)	0.62(Y)	0.88(Y)	
C	0.10(YG)	0.18(BG)	0.26(Y)	0.47(Y)	
T	0.18(YG)	0.32(BG)	0.56(Y)	0.80(Y)	
X	0.12(YG)	0.21(BG)	0.45(Y)	0.70(Y)	

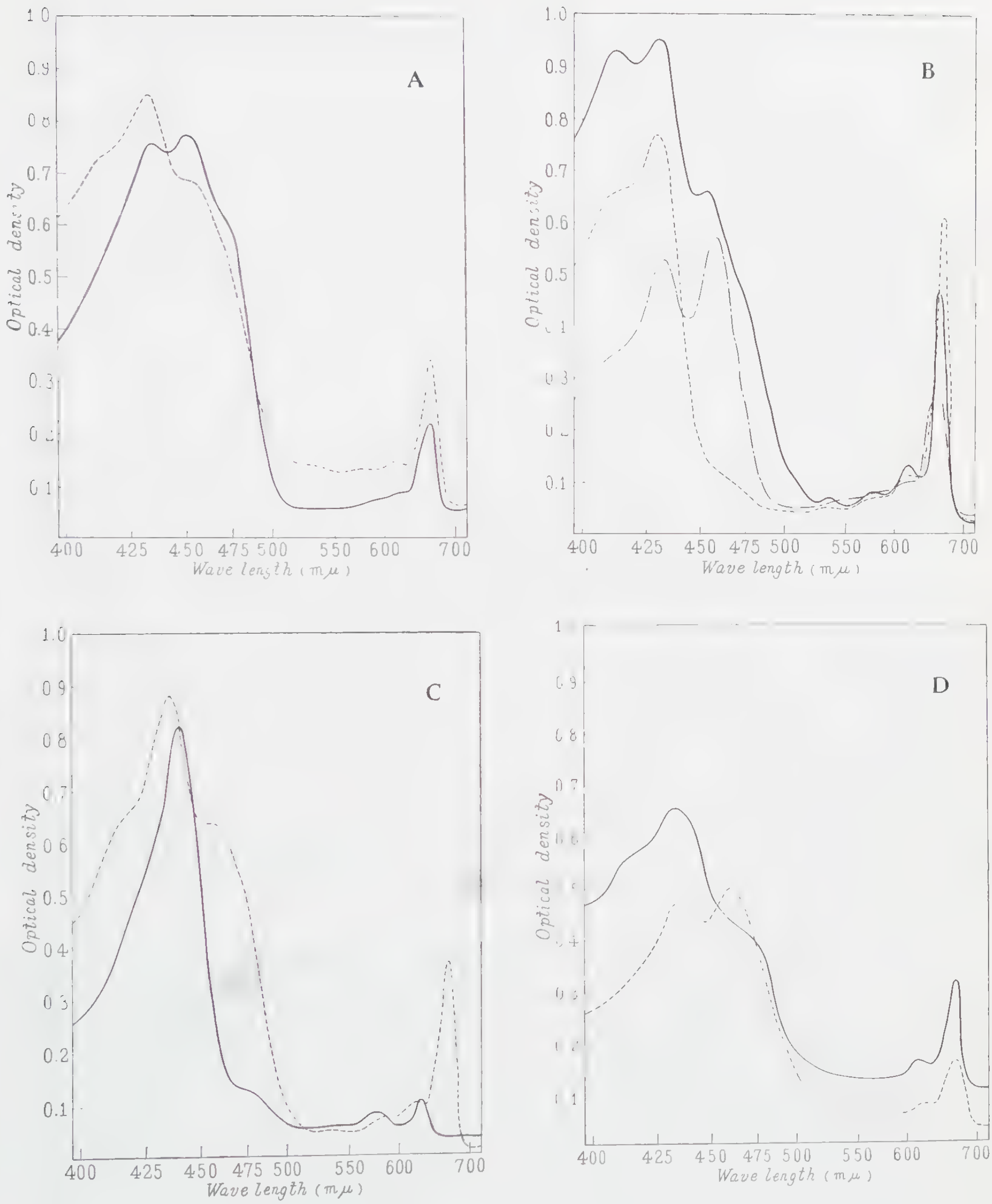

Fig. 2. Absorption curves of the pigmemts dissolved in acetone. A) ———; Plumule of *Nelumbo nucifera*, - - - -; cotyledon of *Pisum sativum*. B) ———; The whole pigment extracted from a normal leaf of *Nelumbo*, - - - -; BG spot after developing the same pigment on a chromatogram. It is chlorophyll *a* to a greater extent. —·—·-; YG spot after developing the same pigment on a chromatogram. It is chlorophyll *b* to some extent intermingled with chlorophyll *a*. C) ———; *Cucurbita maxima*, - - - -; *Citrus Oto*. D) ———; *Citrus nobilis*, - - - -; *Glycine Max*,

The chromatogram of the seed pigments of *C. depressa* showed two spots with carbon tetrachloride, but not with other solvents (Tab. 1 (4)(c)). Moreover the chromatogram of the seed pigments of *C. Keraji* var. *Kabuchii* is not developed by benzene (Tab. 1 (4)(d)).

To identify each spot by paper chromatography, leaf pigments of some plants were also developed, and the results are given in Table 2. Table 2(2) was shown to compare the leaf pigments of *Dolichos Lablab* with that of *N. nucifera*.

In Figure 2, absorption curves of the green pigments are illustrated which were found in the seeds of above mentioned angiospermous plants.

Discussion

Comparing the chromatograms of the plumule with that of the leaf, following conclusions may be drawn; (1) YG spot on the chromatograms is chlorophyll *b*, (2) BG spot, chlorophyll *a*, and (3) Y spot, carotenoid. By these facts, it is evident that the plumule of *Nelumbo* involves chlorophyll *a* and *b*. Y spot of a small Rf value which appeared in the chromatogram of albino leaf of *Dolichos Lablab* (Tab. 2(2)(a)) might be protochlorophyll as a result of comparing it with the chromatograms (b) and (c) in Table 2(2). In view of the chromatogram from the plumule of *Nelumbo*, it is conceivable that the Y spot which appeared near the BG spot should be protochlorophyll, but this awaits further investigation.

In the chromatograms of seed pigments, BG and YG spots were often accompanied by a faint spot of the same tint just following each of them. The tint suggests these spots represent also chlorophyll *a* and *b*. However, the reason why this phenomenon occurs is not yet clarified, but it seems to be overcome by using adequate solvent mixtures.

It is also clear that the cotyledons of *Pisum sativum*, embryos of *Citrus depressa*, *C. Keraji* var. *Kabuchii*, *C. Tachibana* and *Fortunella margarita* contain chlorophyll *a* and *b* (Tab. 1). The seed pigments of *Citrus Oto*, *C. sinensis*, *C. nobilis* and *Glycine Max* showed only YG spots in the chromatograms, but these pigments seem to contain chlorophyll *a* and *b* judging from the absorption curves (Fig. 2-C,-D). The absorption curve of *C. sinensis* was identical with that of *C. nobilis*, so its figure was not shown here. In the chromatograms of the embryos of *C. Junos* and *C, Tamurana* only YG spot appeared. The appearance of the pigment in these cases was not clear because of small quantity.

YG spot in the chromatograms of the seed pigments of *Cucurbita* (Tab. 1(6)) is probably identical with chlorophyll *b*. To ascertain the result, paper chromatography was made using the pigment of the leaf of *C. maxima*. At first spots representing chlorophyll *a* and *b* were cut off by scissors and eluted with the same solvent separately, and mixed with a solution of the seed pigments of *C. maxima*. These two solutions were developed by paper chromatography, respectively. As the results, in

the chromatogram containing chlorodhyll *a* mixed with the seed pigment of *C· maxima* the spots of BG and YG appeared clearly. While in a similar solution containing chlorophyll *b* and the seed pigment, there appeared only YG spot. It is apparant, therefore, that the seed of *C. maxima* contains only chlorophyll *b*. This conclusion agrees well with the absorption curve which is shown in Figure 2-C.

The fruit-coats and seed-coats of *Pisum sativum* and of *Glycine Max* are so thin and semitransparent that it may be supposed they are evidently translucent to the light to some extent. But the plumule of *Nelumbo* and the green tissure of *Cucurbita* are considered to be cut off from the light.

Bogarad[3] noticed chlorophylls formed in the light and in the dark were spectroscopically similar. The writer found chlorophylls which were produced in the seeds seem to differ slightly from those in the normal leaves, especially in *Nelumbo* and *Cucurbita* (see Fig. 2-A, -B and -C).

Acknowledgement

The writer is indebted to Professor S. Hattori of the University of Tokyo for invaluable advice and constant guidance in the course of the work. To Mr. S. Miyachi, Dr. A. Hattori and Mr. Y. Fujita of the Institute of Applied Microbiology, the writer wishes to express cordial gratitude for their operation with the spectrophotometer. Further the writer wishes to express sincere thanks to Professor S. Nagami of the Yokohama University, Professor T. Tanaka of the University of Osaka Prefecture and Honorary Professor Y. Ogura of the University of Tokyo for their kind guidance.

Summary

1. The species of which seed pigments were studied are as follows: *Nelumbo nucifera*, *Pisum sativum*, *Glycine Max*, some species of *Citrus* and *Cucurbita*, and *Fortunella margarita*.

The properties of the green pigments in seeds were examined. These were observed in the plumule of *Nelumbo*, the cotyledons of *Pisum* and *Glycine*, the embryos of *Citrus* and *Fortunella*, and the inner part of the seed-coat of *Cucurbita*.

2. These green pigments were extracted and paper chromatographically studied. The absorption curves of the pigments were also taken with a spectrophotometer.

3. In the seeds of *Nelumbo*, *Glycine*, *Pisum*, some species of *Citrus* and *Fortunella*, both of chlorophyll *a* and *b* were evidently observed, while only chlorophyll *b* was found in the seed of *Cucurbita moschata* and *C. maxima*.

4. The fruit-coat and seed-coat of *Pisum* and *Glycine* seem to be somewhat translucent to the sunlight, while seeds of *Nelumbo* and *Cucurbita* seem to be cut off from the light. None the less chlorophyll *a* and *b* are apparently detected in these seeds.

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摘　　要

1. 種子の中に緑色の物質を有するものがあるが，これらのうち，ハス，エンドウ，ダイズ，ミカン属の数種，キンカンおよびカボチャについて実験した。緑色の物質はハスでは幼芽に，エンドウ，ダイズでは子葉に，ミカン属，キンカンでは胚に，カボチャでは種皮の内側の組織に認められた。

2. これら緑色の物質を抽出してペーパークロマトグラフ法によって分析した。またこれらの数種については分光光度計を用いて吸収曲線を調べた。

3. ハス，エンドウ，ヒラミレモン，*Citrus Keraji* var. *Kabuchii*，タチバナ，キンカンではクロロフィル *a* と *b* を明らかに認めた。また *C. Oto*，アマダイダイ，クネンボおよびダイズでもクロロフィル *a* と *b* を有することが吸収曲線によって明らかにされた。しかし，カボチャではクロロフィル *b* のみが見いだされた。

4. これらの種子のうち，エンドウとダイズの果皮および種皮はうすく，やや半透明で，ある程度日光を透過すると思われるが，ハスとカボチャではおそらく光から遮断されていると考えられる。しかし，これらの種子内の緑色の物質として明らかにクロロフィル *a* と *b* とを認めることができた。

菌類の遺伝学的研究 VII
ウシグソヒトヨの交配系*

武丸恒雄**

Tsuneo TAKEMARU**: Genetical Studies on Fungi VII. Some Problems on the Mating System of *Coprinus macrorhizus* Rea f. *microsporus* Hongo.

1959 年 1 月 6 日受付

Coprinus macrorhizus (ネナガノヒトヨタケ) の交配系については，従来二つの型が報告されている。その一つは Dickson[1] のもので，彼によれば本菌は四極性であるという。これに対して Routien[2] は，同菌の 29 単胞子菌糸体について分析の結果，不規則ながら二極性を示すことを報告している。このように同一の種について二つのたがいに異なる交配系が知られているという事実は，まことに興味ぶかい。*Coprinus* 属の分類はかなり困難であるとされているので，あるいは同じ種名のもとに実は異なる菌をとりあつかったのではないかとの疑問も一応は成りたつであろう。しかしこのような例は分類の比較的容易な種についてもいくつか知られているので[3),4)]，これにはわ何かもっと本質的なものが関与しているように思われる。本菌の 1 forma とみなされるウシグソヒトヨについては，木村詮二氏の多年にわたる精力的な研究があり，その交配系が典型的な四極性であることがすでに報告されている[5]。さらに同氏[6] は，*A* ヘテロ，*B* ホモのいわゆる illegitimate の組合わせのあるもの(全部ではない！)では，二核菌糸がセクターとして出現することを見出した。しかもこの二核菌糸は，担子胞子を豊富にもつ正常な子実体を形成することができるのである。

このように，これらの菌の交配系についてはかなりの不統一と混乱がみられる。そこで筆者はウシグソヒトヨを用いて，これらの基本的な問題ととりくんでみた。その結果，このような不統一と混乱は単にみせかけのものにすぎず，その底には一貫して確固な規則性が存在し，この規則性の上に立てば，上述のまちまちなデータを調和統一して説明しうるのではないかとの知見を得たので報告する。

材料と方法

ウシグソヒトヨは多くの点で *Coprinus macrorhizus* (ネナガノヒトヨタケ) ときわめて類似しているが，ただその担子胞子が後者のものよりもかなり小さいことが注目されるので，本郷次雄氏[7] によってその 1 forma に rank されたものである。本実験には X と d の 2 系統を用いたが，いずれも木村氏が長年供試された系統である。

単胞子分離は西門法[8] によった。発生してきた単胞子起原の菌糸体を，2 個体づつあらゆる組合わせで交配した。この際には，問題の2菌糸体の小片を同一のばれいしょ蔗糖寒天上に 1～2 cm. 離して植えつける (Fig. 1)。これを 30° で培養し，

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* 岡山大学理学部生物学教室植物形態学業績 No. 67. 文科研論文，課題番号 407127. 筆者はこれまで「エノキタケの遺伝学」について VI 報まで発表したが，他の菌類も同時にとりあつかう必要を感じたので，こんごは「菌類の遺伝学的研究」というより大きいテーマのもとに，エノキタケを含めた各種の菌類について研究を続行してゆきたい。

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交配後5〜6日目に検鏡した。検鏡は，まず両菌糸体の接触部の菌糸についてクランプの有無を調べ，次に接触部の両側について同様行なう。この際，0.025% または 0.05% のメチレンブルー水溶液を用いれば検鏡が容易である。本菌は常用のばれいしょ蔗糖寒天上に容易に子実体を形成するが，開傘には光が必要であるため，子実体形成の実験には 30° で明培養を行なった。

被覆四極性

i）クランプ形成能：X 系統に生じた子実体より 22 単胞子を分離し，そのうちの 18 単胞子菌糸体について総あたり交配を行なった。その結果は Table 1a に示された通りである。*A* および *B* 因子がいずれもヘテロであるような和合性組合わせでは，交配された両菌糸体の接触部はもちろんのこと，その両側の菌糸体全面にわたってくまなくクランプの形成がみられた。これを“完全二核化”とよび，表中“十”で示してある。このような二核菌糸体はきわめて粗剛な逞ましい外観を呈するので，クランプを形成しない一核菌糸体とは肉眼で容易に識別することができる。また *A* 因子についてヘテロ，*B* がホモであるような illegitimate の組合わせでは，Fig. 1 で見られるような鮮やかな境界線が，両菌糸体のあいだに形成される。これは Vandendries と Brodie[17] によって“barrage”とよばれた現象であるが，これについてはのちほどあらためて述べることにする。ところで，この境界線の部分には，異常肥大をした菌糸にまじってクランプをもつ細い菌糸が存在することが，本実験によって明らかにされた。菌糸が細いのでそれにともなって形成されたクランプも概して小形であるが，個々のクランプはいずれも真正であって，偽クランプ状の構造は見られなかった*。しかしこの場合には，クランプの形成は境界線にのみ局限され，その両側にはいずれもその形成がみられなかった。したがってこれを“限定二核化”とよび，“(+)”で示した。一方，*A* ホモ，*B* ヘテロの illegitimate 組合わせではこのような現象はまったくみられなかった。

それゆえ，従来行なわれてきたように，クランプ形成の有無を根拠として交配系の型を決定しようとすれば，接触部（あるいは境界線）についてのみ分析された場合には二極性となるであろうし，接触部以外の部位だけについて調査された場合には単なる四極性の交配模様を示すであろう。前述の Dickson[1] や Routien[2] の論文には不幸にして分析の部位が明示されていないが，両者の報告のくいちがいは，あるいはこのような事情に起因するものではなかっただろうか。したがって，クランプ形成の調査は少なくとも接触部とその両側の双方について行なわれるべきであって，その結果を綜合して，いいかえれば単にクランプ形成の有無だけではなくその“状態”をも考慮して交配系の決定を行なうことが必要である。接触部の調査によって二極性の交配模様を得たとしても，調査をさらにその両側にまで及ぼせば，二極性の仮面におおわれていた本来の四極性の姿が浮かび出てくるのである。このような四極性を，筆者は“被覆四極性 masked tetrapolarity”とよぶことにする。

本菌のもう一つの系統 d の 15 単胞子菌糸体についても，上述の X 系統の場合とまったく同様の交配模様が観察された。

Coprinus 属における illegitimate 二核化についてはすでに Brunswik[11] や Vandendries[12,13] などの研究があるが，この場合の二核化が筆者のみた限定二核化であるか，もしくは後述の扇形部二核化として現われたものであるかは不詳である。しかしいずれにしてもこの二核化は，*A* ヘテロ，*B* ホモの illegitimate 組合わせの一部のものに例外的にみられたのであって，本菌の場合のように上記 illegitimate 組合わせの全部において規則正しく営なまれる例は報告されていない。

ii）子実体形成能：X と d 系統を用いた上記実験の際クランプを形成した組合わせの全部について，子実体形成の有無とその状態を調査した。この実験では条件を斉一にするため，交配された両菌糸体の接触部（または境界線）に形成された二核菌糸体を新しい個々の培養基に同時に移植して行なった。このようにして発生した illegitimate 起原の二核菌糸体は，正常の legitimate 起原のものと比べて，なんら遜色のない逞ましい粗剛な外観を呈し，生育も同じようにきわめて旺盛

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* ただし，クランプの排列状態には，和合性組合わせ起原の二核菌糸と上記 illegitimate 起原のものとの間に相異がみとめられたが，これについては別の機会に報告したい。

Table 1. a) Clamp formation from all possible matings between 18 monosporous mycelia from a fruit-body of X stock. b) Fruit-body production from all clamp-positive pairings. Explanation of signs for this and following tables: "+" denotes complete dikaryotization on both sides of the contact zone between two mated mycelia; "(+)", limited dikaryotization found only in the contact zone; "F", perfectly developed fruit bodies bearing abundant basidiospores; "f", half-developed fructification with stipes and undeveloped pilei, no spore being formed; and "—", negative clamp-forming or fruiting reaction.

a

	1	2	10	11	12	17	3	6	8	16	18	5	9	4	7	13	14	15	
1	—	—	—	—	—	—	+	+	+	+	+	—	—	(+)	(+)	(+)	(+)	(+)	A^1B^1
2	—	—	—	—	—	—	—	+	+	+	+	—	—	(+)	(+)	(+)	(+)	(+)	
10	—	—	—	—	—	—	+	+	+	+	+	—	—	(+)	(+)	(+)	(+)	(+)	
11	—	—	—	—	—	—	+	+	+	+	+	—	—	(+)	(+)	(+)	(+)	(+)	
12	—	—	—	—	—	—	+	+	+	+	+	—	—	(+)	(+)	(+)	(+)	(+)	
17	—	—	—	—	—	—	—	+	+	+	+	—	—	(+)	(+)	(+)	(+)	(+)	
3	—	—	+	—	+	+	—	—	—	—	—	(+)	(+)	—	—	—	—	—	A^2B^2
6	+	+	—	[illegible]	—	—	[illegible]	—	—	—	—	(+)	(+)	—	—	—	—	—	
8	—	—	+	+	—	—	—	—	—	—	—	(+)	(+)	—	—	—	—	—	
16	+	+	+	+	+	+	—	—	—	—	—	(+)	(+)	—	—	—	—	—	
18	+	+	+	+	+	+	—	—	—	—	—	(+)	(+)	—	—	—	—	—	
5	—	—	—	—	—	—	(+)	(+)	(+)	(+)	(+)	—	—	+	+	+	+	+	A^1B^2
9	—	—	—	—	—	—	(+)	(+)	(+)	(+)	(+)	—	—	+	+	+	+	+	
4	+	+	+	(+)	+	(+)	—	—	—	—	—	+	+	—	—	—	—	—	A^2B^1
7	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	—	+	+	—	—	—	—	—	
13	(+)	(+)	(+)	(+)	(+)	(+)	—	—	—	—	—	+	+	—	—	—	—	—	
14	(+)	(+)	(+)	(+)	(+)	(+)	—	—	—	—	—	+	+	—	—	—	—	—	
15	+	+	+	+	+	(+)	—	—	—	—	—	+	+	—	—	—	—	—	
	A^1B^1						A^2B^2					A^1B^2		A^2B^1					

b

	1	2	10	11	12	17	3	6	8	16	18	5	9	4	7	13	14	15	
1							F	F	F	F	F			f	F	F	F	F	A^1B^1
2							F	F	F	F	F			F	F	F	F	F	
10							F	F	F	F	F			F	F	f	f	—	
11							F	F	F	F	F			F	F	F	F	F	
12							F	F	F	F	F			F	F	F	F	F	
17							F	F	F	F	F			—	f	—	—	f	
3	F	F	F	F	F	F						f	F						A^2B^2
6	F	F	F	F	F	F						F	f						
8	F	F	F	F	F	F						f	F						
16	F	F	F	F	F	F						F	f						
18	F	F	F	F	F	F						F	F						
5							f	F	f	F	F			F	F	F	F	F	A^1B^2
9							F	f	F	f	F			F	F	F	F	F	
4	f	F	F	F	F	—						F	F						A^2B^1
7	F	F	F	F	F	f						F	F						
13	F	F	f	F	F	—						F	F						
14	F	F	f	F	F	—						F	F						
15	F	F	—	F	F	f						F	F						
	1	2	10	11	12	17	3	6	8	16	18	5	9	4	7	13	14	15	

Table 2. Mating reaction between 4 testers of the original stock and 21 monosporous mycelia from an illegitimate combination of X stock, $5(A^1B^2) \times 6(A^2B^2)$.

Testers	A^1B^2										A^2B^2										
	1	4	5	6	9	12	13	15	16	21	2	3	7	8	10	11	14	17	18	19	20
A^1B^1 (1)	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
A^2B^2 (6)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	−	−	−	−	−	−	−	−	−	−	−
A^1B^2 (5)	−	−	−	−	−	−	−	−	−	−	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
A^2B^1 (7)	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−

である。Table 1b にはX系統についての実験結果だけが示されているが，d 系統の場合にもこれとまったく同じ傾向がみられている。“F”は外観正常で胞子を豊富につけた完全な子実体の発生を示し，“f”は菌柄を生ずるのみで開傘もなく胞子も形成しない場合，“−”は子実体の原基すらも発生しなかった組合わせを表わしている。$A^1B^1 \times A^2B^2$ および $A^1B^2 \times A^2B^1$ の和合性組合わせでは，すべて完全な子実体を形成したが，illegitimate の組合わせ ($A^1B^1 \times A^1B^1$ および $A^1B^2 \times A^2B^2$) においても大部分が正常な子実体を発生している。和合性起原の子実体よりも illegitimate 起原のものの方が一般にいくらか小形であるという傾向を除けば，両者の子実体は外観上区別がつかない。また担子胞子の大きさもほとんどまったく同じである。ただ，子実体形成の状況と所用時間が両者の間でかなり相違している。すなわち，和合性組合わせでは，菌糸体を植えつけてから7～9日後に大部分のものは“一斉に”子実体を形成する。もっとも若干形成がおくれるものもあるが，それでも 12～14 日後には開傘溶解する。これに対して，illegitimate 起原の子実体は，中には 10 日ぐらいで開傘し，和合起原のものと大差ない組合わせもあるが，多くのものは 10～20 日の間にわたって，だらだらと“不ぞろいな”形成を示す傾向がみられた。もっとも時間のかかった例では，28 日後にやっと開傘溶解した子実体もあった。このようにみてくると，illegitimate 起原の子実体の方が，和合性起原のものよりも，その形成がいくらか困難であるように思われる。なお Table 1b および 3b では，一つの組合わせについて各3培養づつ平行培養したものの結果が示されている。

iii) Illegitimate 起原の子実体分析: 和合性組合わせから生じた子実体より分離された単胞子菌糸体を，あらゆる組合わせで交配すれば，ふたたび Table 1a で示されるような被覆四極性の交配模様が得られる。一方，illegitimate の組合わせから生じた F_1-単胞子菌糸体の交配模様はどうなるであろうか。本実験では，和合性組合わせの場合と同程度早くかつ容易に子実体を形成した illegitimate の組合わせ——X 系統の $5(A^1B^2) \times 6(A^2B^2)$ および d 系統の $8(A^2B^1) \times 10(A^1B^1)$ の2例——について F_1 の分析を行なった。X 系統の上記組合わせから由来した 21 F_1-単胞子菌糸体を本系統の四つの交配型を代表するテスタ菌糸体に交配した結果，Table 2 で示されるように，両親と同じ 2 種類の交配型 (A^1B^2 と A^2B^2) だけしか見出されなかった。また d 系統の前記組合わせからも 11 個の F_1-菌糸体を分離したが，この場合にもふたたび両親と同じ二つの交配型が出現しただけであった。ところで単胞子菌糸体の分析個体数があまり少ないと，4 交配型が出現するはずの組合わせでも，たまたまそのうちの2型しか得られないということも起こりうることが考えられる。しかし上述の分析では，X と d 系統を合計すれば 32 単胞子菌糸体について分析したことになり，またいずれの場合にも両親と同じ交配型のものばかりであったことなどからみて，このような心配はまずないと考えられる。

次に X 系統の上記 21 単胞子菌糸体のうち任意の 12 個体について総あたり交配を行なったところ，Table 3a で示されているように二極性の交配模様が得られた。ただしこの場合の二核化は全部限定二核化であって，完全二核化はまったくみられなかった。またこの際に子実体形成能も調査したが，Table 3b で示されているように，今度は子実体の発生が，親系統の illegitimate の組

Table 3. a) Mating pattern between 12 monosporous mycelia of illegitimate origin shown in Table 2. b) Fruit-body production from all pairings in which limited dikaryotization has occurred.

a

		1	4	5	6	9	12	2	3	7	8	10	11
A^1B^2	1	—	—	—	—	—	—	(+)	(+)	(+)	(+)	(+)	(+)
	4	—	—	—	—	—	—	(+)	(+)	(+)	(+)	(+)	(+)
	5	—	—	—	—	—	—	(+)	(+)	(+)	(+)	(+)	(+)
	6	—	—	—	—	—	—	(+)	(+)	(+)	(+)	(+)	(+)
	9	—	—	—	—	—	—	(+)	(+)	(+)	(+)	(+)	(+)
	12	—	—	—	—	—	—	(+)	(+)	(+)	(+)	(+)	(+)
A^2B^2	2	(+)	(+)	(+)	(+)	(+)	(+)	—	—	—	—	—	—
	3	(+)	(+)	(+)	(+)	(+)	(+)	—	—	—	—	—	—
	7	(+)	(+)	(+)	(+)	(+)	(+)	—	—	—	—	—	—
	8	(+)	(+)	(+)	(+)	(+)	(+)		—	—	—	—	—
	10	(+)	(+)	(+)	(+)	(+)	(+)	—	—	—	—	—	—
	11	(+)	(+)	(+)	(+)	(+)	(+)	—	—	—	—	—	—
		A^1B^2						A^2B^2					

b

		1	4	5	6	9	12	2	3	7	8	10	11
A^1B^2	1							—	f	f	—	f	f
	4							—	—	—	—	—	f
	5							—	—	f	f	—	f
	6							—	f	—	—	f	F
	9							f	f	—	f	f	F
	12							f	f	f	f	—	f
A^2B^2	2	—	—	—	—	f	f						
	3	f	—	—	f	f	f						
	7	f	—	f	—	—	f						
	8	—	—	f	—	f	f						
	10	f	—	—	f	f	—						
	11	f	f	f	F	F	f						

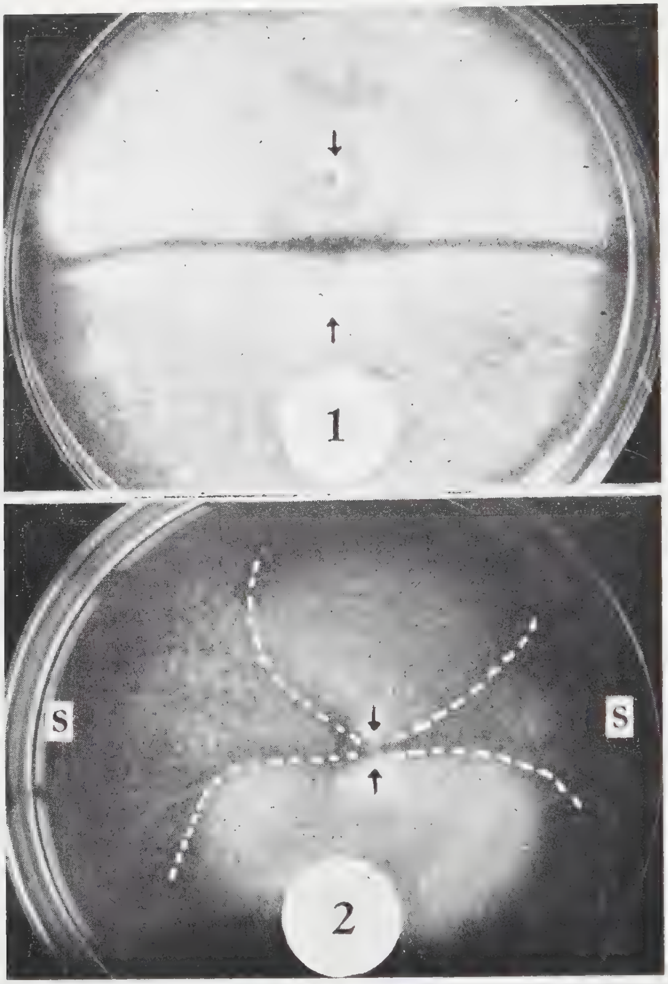
合わせ(Table 1bの$A^1B^1 \times A^2B^1$ と $A^1B^2 \times A^2B^2$)に比較して，きわめて不良であった

illegitimate 起原の子実体分析についてはすでに Oort[9] や Quintanilha[10] などの興味ある業績があるが，これらについては別の機会に述べることにする。

限定二核化と扇形部二核化

二つの単胞子菌糸体を1～2cm. 離して植えつけると，その中間に菌糸の少ない帯状部が形成されて，明瞭な境界線をつくる場合がある (Fig. 1)。これが "barrage" とよばれる現象であることはすでに述べた。ある種の菌では，この barrage は不和合性因子 A および B と一定の関連をもって出現するといわれているが[14)-20)]，このような関連のない菌も報告されている[21)-23)]。*Coprinus* 属においてはつとに Brunswik[11] がこの現象に気づいていたが，その後 Oort[9] は *C. fimetarius* において，2対の不和合性因子のうちの1対が barrage の出現に関係していることを見出した。

さて，ウシグソヒトヨにおける筆者の観察によれば，X 系統ではこの barrage がきわめて鮮明で，その出現も非常に規則的である。すなわち，限定二核化が行なわれる $A^1B^1 \times A^2B^1$ と $A^1B^2 \times A^2B^2$ の illegitimate 組合せでは 100% これの出現がみられるが，他のいずれの組合わせにも現われないのである。しかし d 系統ではいくらかの乱れがみられ，上記の illegitimate 組合わせのほかに，たとえば $A^1B^1 \times A^1B^1$ というような同じ交配型同士の組合わせのあるものにも barrage が



Figs. 1 and 2. Two cultural behaviours in common *E*-factor matings of *Coprinus macrorhizus* f. *microsporus*. Fig. 1. A barrage manifested between two monosporous mycelia. Fig. 2. Sectoring dikaryons (s). Arrow signs indicate inocula. (Natural size).

観察された。また d 系統でみられた barrage の外観は，一般に X 系統の場合ほど鮮明ではなかった。このように，本菌では系統によって barrage の出現様式がいくらか相異しているようである。したがって厳密な意味では，barrage のあるところにはかならず限定二核化があるということはゆるされないが，少なくとも限定二核化のあるところには barrage の出現が伴なうということはいえそうである。

さて前述したように，木村氏[6]は本菌について*A*ヘテロ，*B*ホモの illegitimate 組合わせのあるもの（全部ではない!!）ではセクターをなして二核菌糸体が形成されることを発見した。筆者はかりにこの現象を“扇形部二核化 sectoring dikaryotization”とよぶことにする。同氏によれば，この扇形部二核菌糸は正常な子実体を形成するが，この子実体からは両親と同じ交配型をもつ2種類の胞子しか得られなかったという。

筆者の実験では，すでに「材料と方法」の項で述べたように，二つの菌糸体を1～2cm. の距離で植えつける方法をとったが，その際には限定二核化は barrage に伴なわれていとなまれ，一度も扇形部二核化はみられなかった。そこでとくに木村氏が行なったように，限定二核化をいとなむ

組合わせについて，両菌糸体を“接触して”植えつけたところ，一部の組合わせでは，その移植部を起点とする扇形部に肉眼で明らかに区別できる二核菌糸体を発生した(Fig. 2)。ただしこの扇形部の出現は組合わせによって固定されたものではなくて，むしろチャンスによるものと思われる。というのは，一度扇形部を生じた組合わせでも，2 度目の実験では生じなかったり，またその逆の場合もしばしばみられたからである。一方，境界線に形成された限定二核菌糸を新しい培養基に移植すると，扇形部に発生した二核菌糸体と同じ外観を呈し，クランプの状態もまったく同様の二核菌糸体を形成する。しかもそれから発生した子実体からは，Table 2 でみられるように両親と同じ交配型をもつ2種類の胞子しかつくられないのである。

このようにみてくると，扇形部二核化は実は限定二核化の一つの表現にすぎないものと考えられる。扇形部が出現すること自体は多分にチャンスによるものであるが，illegitimate 二核化そのものは決して偶然でもまた例外でもなくて，限定二核化という一般的なかたちで規則的にいとなまれているのである。

筆者の願いによって心よくネナガノヒトヨタケの系統を御分譲くださった木村氏に深く感謝いたします。

Summary

The mating system of *Coprinus macrorhizus* f. *microsporus* was analyzed, using two stocks X and d. As shown in Table 1a, in matings between monosporous mycelia having unlike incompatibility factors at both loci, clamp-bearing hyphae are observed not only in the contact zone between two mated mycelia but also on both sides of it (complete dikaryotization). Clamps are also found in all matings where the *B*-factors but not the *A*-factors are identical (common *B*-factor mating). In the latter case, however, the formation of clamps is restricted only to the contact zone (limited dikaryotization). Therefore, when only the contact zone is examined for the presence or absence of clamps, a bipolar mating-pattern is obtained. However, when the mycelia on either side of it as well are tested for clamps, tetrapolarity is unmasked. Such tetrapolarity may be called "masked tetrapolarity".

All matings where hyphae with clamps had been observed were tested for their capacity to produce fruit-bodies under the same culture conditions. As shown in Table 1b, perfectly developed fruit-bodies with abundant basidiospores were obtained not only in all matings showing complete dikaryotization but also in some pairings showing limited dikaryotization. Fruit-bodies from the former matings produced spores of all four mating types; whereas, from fruit-bodies formed in the latter pairings, only spores of the two parental types were produced (Table 2). Pairings between the monosporous mycelia of illegitimate origin show bipolar pattern, where only limited dikaryotization, but never complete dikaryotization, regularly occurs (Table 3a).

In common *B*-factor matings, when two mycelia are inoculated 1～2 cm. apart, a clear line of demarcation which is called "barrage" always appears between them, as shown in Fig. 1; whereas, when the two inocula are brought into contact with each other, a sector composed of dikaryotic mycelium develops occasionally in some matings, as shown in Fig. 2. Barrages develop with regular manner in all common *B*-factor matings, but the sectoring dikaryons are rather of haphazard occurrence.

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Short Communication

Eizi OGATA*: **Observations on the Vertical Growth of *Conchocelis***

尾形英二*: コンコセリスの垂直的生長

Received Februry 14. 1959

Observations on the growth of *Conchocelis* filaments in marine shells have been made by the surface view. Scarcely any has so far been known about its growth behaviour in the vertical direction. In this connection, successful observations were made on the *Conchocelis* of *Porphyra tenera* obtained at Yoshimi, Shimonoseki on December 8 and 16, 1958 and grown in our laboratory, appropriately using pearl oyster shells. The following new procedure for preparation enabled us to follow the growth in a whole profile view.

Several rectangular pieces were cut out of pearl oyster shells which harboured the filaments; these pieces were cemented together and glued onto a microscope slide with a sort of synthetic resin; the exposed surfaces were carefully ground extremely thin with a grindstone. Profile of the filaments in these prepared specimens became easily accessible in transmitted light (see the photographs).

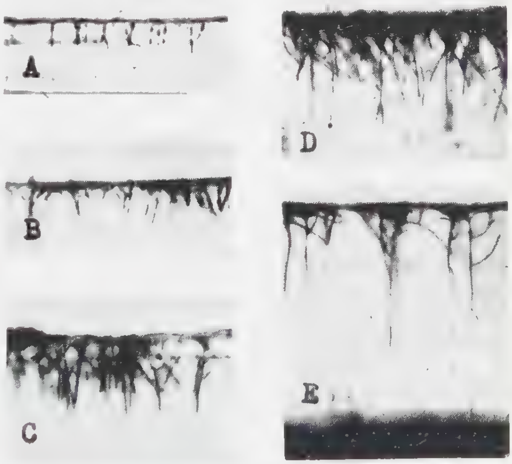

Fig. 1. Profile features of growth in *Conchocelis* filaments. All ×100.

1) The growth in early stages proceeds rather perpendicularly than it was generally assumed before. Most germlings spread tap-root-like filaments downwards into the shell matrix. 2) These main filaments subsequently produce lateral branches which later run upwards and finally reach the shell surface. They somewhat resemble rhizomes in land plants. 3) Thus grown up branch filaments form an anastomosed layer just underneath the shell surface. 4) Main filaments grew up to 20–25 μ in 5 days (A), 50–100 μ in 10 days (B), 100–150 μ in 12 days (C), 150–230 μ in 20 days (D) and 280–350 μ in 30 days (E).

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Tohru HASHIMOTO** and Toshio YAMAKI**: On the Physiological Effects of Gibberelins A_1, A_2, A_3, and A_4*

橋本 徹**. 八巻敏雄**: ジベレリン A_1,A_2,A_3,A_4* の生理作用について.

Received March 5, 1959

It was reported that a mixture of gibberellins A_1 and A_3 promoted the expansion[1] of *Raphanus* leaf and induced the germination[2] of tobacco seeds in the darkness. Here are reported the new findings on the physiological action of gibberellins A_1,A_2, A_3 and A_4, and different effectiveness among them.

The physiological activities of these gibberellins were determined at various concentrations ranging from 10^{-12} to 10^{-4}M. The seeds of *Nicotiana tabacum* L. (Bright Yellow) were sown on filter paper soaked with 0.02 M KNO_3 solution and were allowed to germinate for 5 days at 25° in the darkness. *Phaseolus vulgaris* leaf discs were punched out from the primary leaves of dark grown seedlings and cultured according to Miller's method[3] using two per cent sucrose instead of glucose. *Raphanus* (*sativus* L.) leaf discs were obtained and cultured by Kuraishi's method[4].

All these gibberellins induced in the total darkness the germination of the tobacco seeds, which otherwise failed to take place, and promoted the expansion of *Phaseolus* and *Raphanus* leaf discs. However, the difference in the effectiveness of these four gibberellins is remarkable, especially in inducing the seed germination. The concentrations required for 20 per cent germination were 10^{-4}, 3×10^{-4}, 10^{-4} and 2×10^{-6} for A_1, A_2, A_3 and A_4, respectively, i.e., comparative effectiveness was in the following order, $A_4>A_3\doteqdot A_1>A_2$. In the expansion of leaf discs, too, A_4 was the most effective and its activity was noticed even at as low concentration as 10^{-11}M. Gibberellins A_1, A_2 and A_3 presented complicated relations between concentration and response.

The present data do not correspond to the order[5] of effectiveness of four gibberellins observed in promoting vegetative elongation of epicotyl, flowering of facultative long-day annuals and fruit setting. Detailed report will be presented soon.

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雑　　録

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4. Fukasawa, H. Nucleus substitution and restoration by means of successive back-crosses in wheat and its related genus *Aegilops*.
5. Yoshida, T. Life-cycle of a species of *Batrachospermum* found in northern Kyushu, Japan.
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なお第17巻，第2号の原稿しめきりは，きたる8月末日です。原稿は植物学集報編集委員あてに書留郵便でお送りください。日本植物学集報の原稿の体裁については編集委員会幹事までお問合わせください。

植物学研究連絡委員会の構成は次の通り：

松浦　一（委員長），芦田譲治，芳賀　忞，原　寛，服部静夫，神谷宣郎，木村有香，三輪知雄，門司正三，小倉　謙，大政正隆，小野記彦，島村環，下斗米直昌，白石代吉，田中信徳（幹事），田杉平司，和田文吾

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本会記事

（役員移動）

（会長選挙）

会則第9条および付則第3第1条によっておこなわれた会長改選の投票は，2月末日に締切り，翌3月1日開票いたしました。その結果，現会長の服部静夫氏が三選され，36年3月31日まで会長の任にあたられます。

開票の結果は次のとおりです。

服部静夫	288
本田正次	217
和田文吾	83
その他	29
合計投票数	617

なお今回の選挙の投票率は非常に良好でした。会員各位のご協力に感謝いたします。

（評議員選挙）

会則第8・9条および付則第3第2条によって，各支部ごとにおこなわれた評議員選挙の結果は次のようになりました。

北海道支部（定員2名）
宇佐美正一郎，松浦　一

東北支部（定員2名）
神保忠男，長尾昌之

関東支部（定員8名）
大槻虎男，原　寛，湯浅　明，高宮　篤，和田文吾，林　孝三，宝月欣二，小野記彦

北陸支部（定員2名）
[illegible]

中部支部（定員2名）
島村　環，田中　潔

近畿支部（定員4名）
北村四郎，三木　茂，小清水卓二，神谷宣郎

中国四国支部（定員3名）
辰野誠次，鈴木兵二，木村劼二，

九州支部（定員3名）
細川隆英，野口　彰，瀬川宗吉

なお任期は34年4月1日から2ヵ年です。

近畿支部

昭和34年度第2回例会（2月7日於京大・理・植物）

広江美之助：アジア産セリ科植物の分類と地理

[illegible]について

*熊野　茂・広瀬弘幸：日本淡水産褐藻の一種 *Heribaudiella fluviatilis* (Areschoug) Svedelius の遊走細胞と生殖器官についての二，三の観察（*印は演者）

投稿についての注意

最近すりあがりが8ページをこえる長論文が多くなっております。長論文が多くなりますとページ数の関係から掲載できる論文数に制限をうけ，ひいては掲載の時期もおくれることになりますので，文章はできるだけ簡潔に書いて，制限ページをこえないよう御注意ください。

また，著者校正のさい，かなり長い補追をされるかたがありますが，投稿規定にもありますように，特別の事情がない限り，[illegible]ていますので，じゅうぶん御承知ください。

Studies on the Light Controlling Photoperiodic Induction of *Pharbitis Nil*. II. Effect of Far-red Light Preceding the Inductive Dark Period

by Atsushi TAKIMOTO* and Katsuhiko IKEDA*

滝本 敦*・池田勝彦*: アサガオの花芽形成を支配する光条件について,
II. 暗期前に与えた近赤外光の影響

Received January 17, 1959

It has been reported in a previous paper that *Pharbitis* seedlings fail to flower if 8 hours of incandescent light of low intensity precedes the inductive dark period and that this flower inhibitory effect of the incandescent light is attributable to the action of the far-red light included in it[1]. Recently Nakayama reported a flower inhibitory effect of far-red light given just before the dark period in *Pharbitis* seedlings of a strain which differs from our material[2]. A flower promoting effect of far-red light preceding the dark period was reported by Borthwick *et al.* in *Xanthium saccharatum*[3]. In order to find the cause of these quite opposite results, the present investigation was undertaken.

Material and Methods

Seedlings of *Pharbitis Nil*, strain "Violet", were used as material. The experimental methods employed were similar to those reported in a previous paper[1].

Experimental results

1) Effect of far-red light preceding the dark period upon flowering responses.

i) Plants were exposed to far-red light of 60 kiloerg/cm.^{2}/sec. (700–1000 mμ) for various hours and subsequently given a 16-hour dark period, which is fully effective for floral induction.

Results represented in Table 1 indicate that the far-red light prevented floral initiation. Experiments were performed several times with similar results. The flower inhibitory effect of the far-red light increased with increasing duration of the irradiation and became striking with 8-hour irradiation.

ii) Plants were exposed to far-red light of 60 kiloerg/cm.^{2}/sec. for various hours and subsequently given a 12-hour dark period, which is not so inductive as to bring

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Table 1. Effect of far-red light preceding a 16-hour dark period upon flower initiation of *Pharbitis* seedlings.

Intensity of far-red light: 60 kiloerg/cm.^{2}/sec.
(Treated on Nov. 26, and dissected on Dec. 23, 1957)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
$16^{h}d$	18	100	4.8	100
$5'FR \rightarrow 16^{h}d$	22	100	3.5	31.8
$1^{h}FR \rightarrow$ 〃	30	93.3	2.1	0
$2^{h}FR \rightarrow$ 〃	42	95.2	2.2	0
$4^{h}FR \rightarrow$ 〃	38	97.4	2.3	0
$8^{h}FR \rightarrow$ 〃	38	8.0	0.1	0

$5'FR \rightarrow 16^{h}d$: 16-hour dark period preceded by 5 minutes' far-red light.
$1^{h}FR \rightarrow 16^{h}d$: 16-hour dark period preceded by 1-hour far-red light.
These abbreviations will be used hereafter.

about the maximum flowering responses. Experiments were repeated several times, and gave similar results in all cases. Some of the results are shown in Table 2. An unexpected result is the flower promoting effect of the far-red light of short

Table 2. Effect of far-red light preceding a 12-hour dark period upon flower initiation of *Pharbitis* seedlings.

Intensity of far-red light: 60 kiloerg/cm.^{2}/sec.
(Treated on May 9, and dissected on May 26, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
$12^{h}d$	21	14.3	0.1	0
$30'FR \rightarrow 12^{h}d$	29	51.7	0.6	0
$2^{h}FR \rightarrow$ 〃	26	96.2	1.6	0
$4^{h}FR \rightarrow$ 〃	28	100	1.8	0
$8^{h}FR \rightarrow$ 〃	29	0	0	0

duration. When the duration of the far-red irradiation was less than 4 hours, flowering was promoted in all cases. But if the far-red irradiation was given for 8 hours it inhibited flowering completely. Why the far-red light of 4 hours or less inhibits flower initiation when followed by a 16-hour dark period, but promotes when followed by a 12-hour dark period will be discussed later.

iii) Plants were exposed to 8 hours of far-red light of various intensities and subsequently given a 16-hour dark period. Results are presented in Table 3. All plants which were not exposed to the far-red light initiated terminal flower buds, whereas the plants exposed to 8 hours of far-red light of 100–5000 erg/cm.^{2}/sec. initiated no flower bud. Even with only 10 erg/cm.^{2}/sec., flowering was inhibited heavily; that is, only 78 % of the plants initiated flower buds, and no plant initiated

Table 3. Effect of the intensity of far-red light preceding a 16-hour dark period upon flower initiation of *Pharbitis* seedlings. Far-red light of various intensities was given for 8 hours preceding the 16 hour dark period.

(Treated on June 17, and dissected on July 3, 1958)

Energy of far-red light in erg/cm.²/sec.	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
5000	40	0	0	0
1000	40	0	0	0
400	39	0	0	0
200	40	0	0	0
100	40	0	0	0
50	39	7.7	0.1	0
20	40	30.0	0.3	0
10	41	78.0	0.9	0
Control				
$16^{h}d$	39	100	4.1	100
$24^{h}d$	40	100	4.7	95.0

a terminal flower. Thus far-red light of very low intensity inhibits flowering if it precedes the inductive dark period.

2) Effect of red light preceding the inductive dark period.

Plants were exposed to red light of 3000 erg/cm.²/sec. for various hours, and subsequently given a 16- or 12-hour dark period. Results are shown in Table 4.

Table 4. Effect of red light preceding 12- and 16-hour dark periods upon flower initiation of *Pharbitis* seedlings.

Intensity of red light: 3000 erg/cm.²/sec.
(Treated on Jan. 15, and dissected on Feb. 7, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
$16^{h}d$	22	100	3.9	77.3
$1'R \rightarrow 16^{h}d$	21	100	4.4	100
$5'R \rightarrow$ 〃	21	100	4.4	90.5
$30'R \rightarrow$ 〃	20	100	3.9	60.0
$2^{h}R \rightarrow$ 〃	21	100	4.2	66.7
$4^{h}R \rightarrow$ 〃	20	100	4.4	95.0
$12^{h}d$	18	50.0	0.5	0
$1'R \rightarrow 12^{h}d$	21	38.1	0.5	0
$5'R \rightarrow$ 〃	23	8.7	0.1	0
$30'R \rightarrow$ 〃	20	30.0	0.3	0
$2^{h}R \rightarrow$ 〃	20	65.0	0.8	0
$4^{h}R \rightarrow$ 〃	22	77.3	1.0	0

R: red light

The red light has little effect on floral initiation whether a 16- or 12-hour dark period follows. Similar results were obtained in several repetitions of the experiment.

3) Reversible effect of red light on the flower inhibitory effect of far-red light preceding the inductive dark period.

Plants were exposed to far-red light of 60 kiloerg/cm.^{2}/sec. for 8 hours and subsequently given 0, 1, 5, 30 and 120 minutes of red light of 3000 erg/cm.^{2}/sec. followed by a 16-hour dark period.

Table 5. Reversible effect of red light upon flower inhibition of far-red light preceding the inductive dark period.

Intensity of far-red light (FR): 60 kiloerg/cm.^{2}/sec.
Intensity of red light (R): 3000 erg/cm.^{2}/sec.
(Treated on Apr. 18, and dissected on May 7, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16^{h}d	20	100	4.8	100
8^{h}FR ——→ 16^{h}d	21	9.5	0.1	0
〃 —→1′R—→ 〃	20	70.0	1.0	0
〃 —→5′R—→ 〃	19	100	2.9	15.8
〃 —→30′R—→ 〃	18	100	2.6	5.6
〃 —→2^{h}R—→ 〃	14	92.9	2.1	7.1

As shown in Table 5, the red light can reverse the flower inhibitory effect of the far-red light, but not completely. As little as 1 minute of red light is effective to some extent. As has been reported in a previous paper, a similar reversible effect of red light was observed for the flower inhibitory effect of incandescent light of low intensity preceding the inductive dark period[1)].

4) Effect of incandescent light of low intensity preceding the inductive dark period.

From the experiments mentioned above and those reported previously[1)], it is noticed that the flower inhibitory effect of far-red light of high intensity bears a remarkable similarity to that of incandescent light of low intensity (10 lux) when followed by a 16-hour dark period. To make more detailed comparisons between them, the following experiments were done.

i) One group of the plants was exposed to far-red light of 60 kiloerg/cm.^{2}/sec. for 5 min., 30 min., 2 hr., 4 hr. and 8 hr., another group exposed to incandescent light of 10 lux for the same durations, and subsequently both groups were given a 16-hour dark period. Results are shown in Table 6. Both far-red and incandescent light inhibited flowering but the former more heavily so than the latter. Both lights, however, inhibited flowering completely when administered for 8 hours.

ii) Low intensity incandescent light (10 lux) was given to plants for 2, 4, 8,

Table 6. Similarity of the far-red and the incandescent light of low intensity with respect to their flower inhibitory effect when followed by an inductive dark period in *Pharbitis* seedlings.

Intensity of far-red light (FR): 60 kiloerg/cm.²/sec.
Intensity of incandescent light (IL): 10 lux.
(Treated on Apr. 22, and dissected on May 10, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16hd	37	100	5.4	100
5′FR→16hd	38	89.5	2.1	5.3
30′FR→ 〃	34	91.2	2.1	8.8
2hFR→ 〃	37	48.6	0.8	0
4hFR→ 〃	37	43.2	0.5	0
8hFR→ 〃	34	0	0	0
5′IL→ 〃	35	100	5.5	91.3
30′IL→ 〃	38	100	4.6	71.1
2hIL→ 〃	36	100	5.0	86.1
4hIL→ 〃	36	97.2	3.3	33.3
8hIL→ 〃	35	0	0	0

12, 16 and 24 hours and followed by 12-hour darkness in one group, and by 16-hour darkness in another one. Results presented in Table 7 show the remarkable flower-promoting effect of the incandescent light given for 4 hours or less preceding the

Table 7. Effect of incandescent light of 10 lux (IL) preceding 16- and 12-hour dark periods upon flower initiation of *Pharbitis* seedlings.

(Treated on May 3, and dissected on May 19, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16hd	39	100	3.7	100
2hIL→16hd	40	100	4.2	100
4hIL→ 〃	40	100	4.0	100
8hIL→ 〃	39	94.9	1.6	0
12hIL→ 〃	37	89.2	1.3	0
16hIL→ 〃	37	81.1	1.0	0
24hIL→ 〃	39	79.5	1.1	0
12hd	40	47.5	0.6	0
2hIL→12hd	37	100	4.6	43.2
4hIL→ 〃	38	100	4.4	78.9
8hIL→ 〃	39	2.6	0.0	0
12hIL→ 〃	40	0	0	0
16hIL→ 〃	39	0	0	0
24hIL→ 〃	40	0	0	0

12 hour dark period. This phenomenon had also been observed with the far-red light of 60 kiloerg/cm.^{2}/sec. but with less flower promotion (cf. Table 2). When the low-intensity incandescent light was given for 8 hours, flowering response was suppressed strikingly whether the following dark period was 12 or 16 hours. The same effect had been found for far-red light of 60 kiloerg/cm.^{2}/sec. for 8 hours. Further increase in length of the low-intensity incandescent light had little effect on floral inhibition.

5) Effect of daylight fluorescent light of low intensity preceding the inductive dark period.

As low-intensity light of the incandescent lamp and the far-red light of high intensity showed a similar effect upon flowering responses when given prior to the inductive dark period, low intensity light of a daylight fluorescent lamp comprising little far-red light was investigated in this experiment for its effect on flower initiation when given to the plant preceding the 16- and 12-hour dark periods.

Plants were exposed to the daylight fluorescent light of 10 lux for 0, 2, 4, 8, 16 and 24 hours, and subsequently given 16- or 12-hours of darkness. Flowering responses are shown in Table 8. The fluorescent light preceding 16-hour dark

Table 8. Effect of low-intensity light of daylight fluorescent lamp (10 lux) (FL) preceding 16- and 12-hour dark periods on flower initiation of *Pharbitis* seedlings. (Treated on May 5, and dissected on May 21, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16hd	37	100	4.1	100
2hFL→16hd	37	100	4.3	100
4hFL→ 〃	38	100	4.2	100
8hFL→ 〃	38	100	3.8	63.2
12hFL→ 〃	39	100	4.0	64.1
16hFL→ 〃	40	100	3.1	37.5
24hFL→ 〃	38	100	4.3	92.1
12hd	38	79.0	1.4	0
2hFL→12hd	37	100	4.2	51.3
4hFL→ 〃	38	100	4.3	100
8hFL→ 〃	38	100	3.4	44.8
12hFL→ 〃	39	76.9	1.3	0
16hFL→ 〃	36	94.5	1.8	13.9
24hFL→ 〃	39	89.8	1.6	0

period inhibited the flowering to some extent if the duration of the irradiation was increased up to 8 hours or more, but the inhibitory effect was far less than that of far-red light. In this case, too, further increase in the length of irradiation beyond 8 hours has little effect, the inhibitory level remaining about the same (cf. Table 7). Several other experiments showed similar results.

Daylight fluorescent light of low intensity promoted flower initiation more strikingly than the incandescent light when followed by the 12-hour dark period, and even if the duration of irradiation was increased up to 8 hours, flower initiation was promoted.

Discussion

In the foregoing experiments far-red light preceding a 16 hour dark period inhibited flower initiation, but that of less than 4 hours given prior to 12-hour dark period promoted flowering. Nakayama reported recently a flower inhibitory effect of far-red light given prior to a 16-hour dark period in *Pharbitis* seedlings[2]. This is also the case in our experiments reported here, in which the duration and the intensity of far-red irradiation differs from that in Nakayama's experiment considerably. In his experiment, 2 minutes of far-red irradiation of high intensity resulted in the complete suppression of flowering. The material of his experiment seems to be not so sensitive to the dark period as ours. His experimental treatments were repeated 3 times, whereas in our experiments only one treatment was given. The differences between the present results and Nakayama's may be due to the difference of photoperiodic sensitivity of the material used.

Borthwick *et al.* reported with *Xanthium* the flower-promoting effect of far-red light given prior to the dark period, resulting in a reduction of critical dark length[3]. This may correspond to the flower-promoting effect of the far-red light preceding the 12-hour dark period in *Pharbitis* seedlings, but the flower-promoting effect reported by Borthwick *et al.* appears more striking than that represented here.

Why does far-red light of 4 hours or less preceding a 16-hour dark period prevent flowering, and the same light preceding a 12-hour dark period promote flowering? The results may be elucidated plausibly by the following assumptions.

1) The first partial process taking place in the inductive dark period can proceed under far-red light as well as under darkness or low-intensity fluorescent light[4].

2) Far-red light preceding the inductive dark period brings about changes or conditions which make the following dark period ineffective. This inhibitory action is not effective in the first 4 hours but becomes so after some 8 hours.

Thus far-red light given prior to the dark period has dual effects: the first effect is promoting and the second inhibitory to the flowering responses.

Pharbitis seedlings are induced to flower only to a slight exent when subjected to a 12-hour dark period, but induced to a maximum with a 16-hour dark period, and the further lengthening of the dark period can increase flowering responses.

If the 12-hour dark period is preceded by 4 hours of far-red light, during which the first process of the dark period proceeds, the flowering response corresponds to that induced by 16-hour dark period; that is, in flower promotion. As the far-red light, on the other hand, prevents the subsequent dark process, the overall response is determined by the difference between the flower promoting and inhibiting effect

of the far-red light. In this case, the promoting effect exceeds the inhibitory one, and results in a flower promotion. When the 12-hour dark period is preceded by 8 hours of far-red light, the flower inhibitory effect exceeds the promoting one, and results in a flower inhibition.

If the 16-hour dark period is preceded by 4 or 8 hours of far-red light, even if the first process of the inductive dark period proceeds under this light, flowering may not be promoted, because the 16-hour dark period is sufficient to induce maximum flowering responses, and further extension of the dark period has no further effect. In these cases, only the flower inhibitory effect of the far-red light appears, and results in a flower inhibition.

The above hypothesis for the action of the far-red light preceding the dark period is also applicable to that of the incandescent light of low intensity, which includes abundant far-red light. As a whole, the incandescent light of low intensity has less flower inhibitory effect than the far-red light of high intensity, and has more flower promoting effect than the latter when followed by a 12-hour dark period.

When the low intensity light of a daylight fluorescent lamp, which includes little far-red light, precedes the 12-hour dark period, the above mentioned flower-inhibitory effect of the far-red light can be neglected; therefore, pronounced flower promotion is the result, even if the irradiating duration exceeds 8 hours.

If the daylight fluorescent light of low intensity was given for 8 hours or more preceding the 16-hour dark period, flowering was inhibited to some extent, as revealed in the percentage of plants with terminal flower in Table 8. Similar results were reported in the previous paper[1], and it was supposed that this inhibitory effect of low intensity light of longer duration might be due to the lack of photosynthates.

It is interesting that the flower inhibitory effect of far-red light of high intensity, and of incandescent and daylight fluorescent light of low intensity increases abruptly when the irradiating duration is extended up to 8 hours, and further extension has little effect. Under these lights, the first process of the flower-inducing dark period is supposed to proceed, and some critical changes may take place after some 8 hours.

Summary

1) Far-red light given to *Pharbitis* seedlings preceding a 16-hour dark period inhibits flowering. This flower inhibitory effect is intensified with increasing duration of the far-red irradiation and becomes marked with 8-hour irradiation.

2) The flower inhibitory effect of the far-red light is obvious even if the intensity is decreased to 10 erg/cm.^{2}/sec.

3) Far-red light of 2–4 hours preceding a 12-hour dark period promotes flowering, but the same light of 8 hours inhibits completely.

4) Red light preceding the inductive dark period has no influence upon flowering response whether a 16- or 12-hour dark period follows.

5) The flower inhibitory effect of far-red light preceding the inductive dark period can be reversed by red light applied just before the dark treatment.

6) Incandescent light of 10 lux promotes flowering remarkably when applied for 2-4 hours preceding the 12-hour dark period, but inhibits when given for 8 hours or more.

7) Daylight fluorescent light of 10 lux which includes little far-red light promotes flowering remarkably when preceding the 12-hour dark period.

A discussion was given of the possible role of far-red light and low intensity light preceding the inductive dark period in flowering responses.

Grateful acknowledgment is given to Prof. S. Imamura for his suggestions and criticisms.

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摘　　要

1) アサガオの子葉に 16 時間の暗期を与える前に，近赤外光 (FR) を与えると花芽形成が抑制される。しかし 12 時間の暗期前に FR を与えると，その照射時間が 4 時間以下の場合のみ花芽形成の促進がみられる。

2) 暗期前に与えた赤色光 (R) は花芽形成に対してほとんど影響を及ぼさない。

3) 暗期前に与えた FR の花芽形成抑制効果は暗期直前に R を与えることにより消却される。

4) 10 ルックスの FR を多く含む白熱電灯光 (IL) も FR と同じような効果を示すが，FR よりも花芽形成促進効果が強く，抑制効果が弱い。

5) 10 ルックスの FR をほとんど含まない昼光色螢光燈光 (FL) を 12 時間暗期前に与えるといちじるしく花芽形成が促進される。同じ光を 16 時間暗期前に与えると，照射時間が長い場合のみ，わずかに花芽形成が抑制される。

6) 暗期前に与えた FR, IL および FL の作用に関し二，三の考察を行なった。

The Distribution of Laticiferous Tissue in Different Parts of *Regnellidium diphyllum* Lindman

by M. DURAIRATNAM*

M. Durairatnam*: *Regnellidium diphyllum* Lindm. の器官における乳組織の分布

Received December 15, 1958

Regnellidium is a monotypic genus of the family Marsileaceae found in Brazil, having extremely restricted distribution. It occurs only in the southern part of Brazil. Curiously enough the fossils of this peculiar member of the Marsileaceae are found in the Deccan Intertrappeans in India in places such as Mahgaon Kalan in the Madhyapradesh and at Vikarabad in Hyderabad State. Apart from these unique features in the morphology of the plant and its discontinuous geographical distribution the plant possesses several interesting characters worthy of further investigation. One such character, which proved to be so far unrecorded in ferns, was observed by Mahabale[1)] namely the occurrence of laticiferous tissue. After a preliminary survey he was good enough to suggest to me to find out the distribution of this tissue and handed the material to me for investigation. The present part of the paper gives an account of the observations made in this direction on the distribution of this tissue in different parts of the plant with the help of the material available. Further work on the ontogeny and development of this tissue and its comparison with the fossil species is being worked out by Mahabale. A single sporocarp that developed in the clone that was being reared by Mahabale was obtained when matured. But by this time the sporocarp has become so thick that it was not possible to get complete sections of it. It was therefore cut into smaller bits to see, if the laticiferous tissue occurred in it. The sections were stained in safranin and light green or with Delafield's haematoxylin and light green. Because of its chemical nature and denser protoplasm, the laticiferous tissue stands out clearly from the surrounding parenchymatous cells, but in young parts the latex being watery gets dissolved in acetic alcohol, and leaves merely brown coloured cavities in the section.

Observation and general remarks

A longitudinal section through a growing point clearly shows that some of the cells in the periblem become larger than the rest and lie in the vertical rows. They retain their nuclei for a long time and turn brown. The cells get elongated and sometimes the partition walls are lost. The structure becomes a continuous tube containing a mass of coenocytium.

In a transverse section of the adult stem the laticiferous tissue is mainly distributed in the form of epidermal glands and as tubes in the sub-epidermal cells

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in the parenchyma of the outer cortex, phloem, and the parenchyma of the central pith. In an old stem, toward the end of the season especially, the protoplasm of the laticiferous tissue increases more in extent in the outer cortex and in the central pith. The colour of these cells also changes from light to deep brown suggesting thereby that latex gets concentrated. In transverse section the laticiferous tissue is outlined by four or five cells resembling epithelium in the midst of which there is a thin tube. It continues vertically upwards and downwards for a short distance and form a lattice pattern.

In a transverse section of the petiole the laticiferous tissue occurs in the hypodermis, in the inner cortex, in the midst of the phloem parenchyma, inside the stele and in the form of epidermal glands. Here some of the laticiferous tubes are seen in the radiating strands connecting the vascular bundles with the outer cortex. They end blindly in the diaphragm which occurs in plenty in the air cavity of the petiole.

The cells containing latex in the epidermis of the petiole, stem, and leaf, are small ovate disc-shaped glands having a stalk cell lodged in hypodermis and a globular or disc-shaped elongated cell containing latex. Inside the leaf there are no laticiferous glands or long coenocytic tubes, and the latex is rather diffused in the form of acrid juice and is not very well defined either chemically or histologically. But due to its presence (in the form of small particles on the surface of the leaf) it becomes shining and highly resistant to the action of water. No laticiferous tissue was detected in the root.

I regret that no material of the sporocarp or its stalk was available and the only part that could be satisfactorily studied was the wall of the sporocarp. Here it will be seen that the laticiferous tissue occurs below the layer of tublar cells in the mass of parenchyma lying between the placental layer forming septa and in the middle layers in the wall of the sporocarp. These cells look deep brown and are scattered in large numbers in the spongy parenchyma of the wall of the sporocarp.

It thus appears that the laticiferous tissue in this plant is made up of two component: (1) obovate disc-shaped, latex secreting glands in the epidermis and (2) long, vertically elongated irregularly spread and interconnected laticiferous tubes in the outer and inner cortex, in the cells of the aerenchymatous diaphragm, in the adjoining phloem, and in the central parenchyma inside the stele. Its distribution does not seem to be very fixed. It appears to be the same type as in some of the members of the Musaceae, Aroideae and Euphorbiaceae. Unlike the euphorbiaceous plants it contains no storage of starch grains.

The laticiferous tissue in ferns is a novelty which has not been reported so far. There is a strong probability that is may now be found in some other members of the Filicales and on account of its highly specialized nature may prove to be a very reliable character for comparisons. It has been shown by Jeffrey[3] that one can profitably use this character for the isolation of sub-families in the Compositae. It can also be used for the isolation of some genera as in the Convolvulaceae, or for

the identification of species of aroids. In the family Compositae, the sub-families Tubiflorae and Liguliflorae are distinguished from each other, among other characters by the presence of laticiferous tissue in the former. In the sub-family Cynerae there is no latex, but it only occurs in the bundles of the conservative floral parts. In the Convolvulaceae most of the genera possess laticiferous tubes or acrid juice, but there are no laticiferous canals in *Cressa cretica*. On the other hand, sometimes this character is only of specific importance. For example, in species of *Anthurium*, sometimes the latex accumulated in the cells of the aerenchymatous diaphragm so as to form a distinct spot. Generally the latex is stored above the diaphragm in the cavities. In some species of *Cyclanthus*, e. g. *C. biparitus*, latex is very clear especially in old leaf petioles, but in *Cyclanthus cristatus* there is mostly acrid juice. What is the state of the laticiferous tissue in *Regnellidium diphyllum* is yet a matter for further investigation in comparison with the other two genera of the Marsileaceae. But it is evident from the example that the presence of laticiferous tissue in *Regnellidium* is a character of much wider significance in the Filicales than hitherto understood. Our knowledge about its occurrence is at present restricted to *Regnellidium diphyllum* and needs to be extended to other ferns.

In conclusion I wish to state that this work was carried out at the Royal Institute of Science, Bombay University and my thanks are due to Dr. T. S. Mahabale, Professor of Botany, Poona University, Poona 7, India for allowing me to work on this plant and for giving me the valuable material of this rare plant and for his guidance.

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摘　　要

Mahabale (1950) はデンジソウ科の *Regnellidium diphyllum* ではじめて乳組織を観察したが，筆者はさらに諸器官における乳組織の分布について観察した。切片はサフラニンとライトグリーンか，またはヘマトキシリンとライトグリーンで染めた。

大きくなった茎の乳組織は，表皮では主に毛の形で，また下表皮細胞，皮層の外側，篩管，髄の柔組織中では管の形で分布している。古い茎においては，外皮層，髄で乳組織の原形質の増加がみられ，これらの細胞の色もまた淡褐色から濃褐色に変わる。横断面における乳組織はエピセリウム状の 4～5 細胞の中央に位置し，これらの乳組織は上下に向かって短かく伸び，格子模様を形成する。

葉柄の横断面における乳組織は，下表皮，皮層の内側，篩管柔組織中，中心柱内に存在し，また表皮では腺の形で見出される。乳組織のあるものは，外皮層と維管束を放射状に結んでおり，それらは通気組織の横隔内で終っている。乳組織は根では見出されなかった。要するに，この植物における乳組織は2つの種類からできている。

1) 表皮の倒卵形の盤状細胞と　2) 皮層の内・外層，横隔の細胞，篩部，中心柱内にある柔組織における，長くて垂直的に不規則に延びて連なった乳管とである。しかし，それらの分布は厳密に決ったものとは思われない。

（野津良知）

Chlorophyll Content and Primary Production of the Kuroshio off the Southern Midcoast of Japan

by Shun-ei ICHIMURA* and Yatsuka SAIJŌ**

市村俊英*・西条八束**： 本邦南方黒潮海域のクロロフィル含量と基礎生産

Received February 18, 1959

During the last decade, the study of the organic matter production by phytoplankton in the ocean has been rapidly advanced by many investigators. Especially, C^{14} tracer technique introduced by Steeman Nielsen[1] gave us the most sensitive and convenient method for the measurement of primary production. By means of C^{14} carbonate, the measurement of primary production in the Pacific Ocean was first undertaken by Steemann Nielsen and Abye Jensen[2], and after that several researches have been made in the same way by investigators as Doty and Oguri[3),4)], Jitts[5], and Holmes *et al.*[6]. However, most of these investigations were concerned with the middle and south Pacific, and the data regarding the north-western Pacific, especially about the adjacent sea of Japan, are currently very scarce. We can quote only the studies by Miyake[7] in the area off the Kii Peninsula and some researches by Sorokin[8] in the adjacent sea of Hokkaido.

Recently, the authors have made researches on the primary production in the north-western Pacific Ocean. Practically, the production was determined by the C^{14} method and the daily rate of primary production was estimated from chlorophyll amount, light penetration, and incident radiation as made by Ryther and Yentsch[9].

The authors have reported in this paper the results obtained in August 1957 and in May 1958 during the cruises in the Kuroshio area.

Cruising Courses and Methods

The cruising courses of the research vessel "Takuyo" are shown in Fig. 1, in which the observation station, surface water temperature and direction of the Kuroshio main stream are plotted. The first investigation was made during August 1–18, 1957 in the southern area off the midcoast of Japan as far as lat. 30°N and the second during May 1–9, 1958 as far as lat. 32°N in the same area. The surface water temperature in August were 20° to 28° and the transparency was observed 30 m. or deeper by means of the Secchi disk. On the other hand, the temperature during

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the 1958 cruise indicated a range of 17°–23° and the transparency ranged from 12 to 25 m..

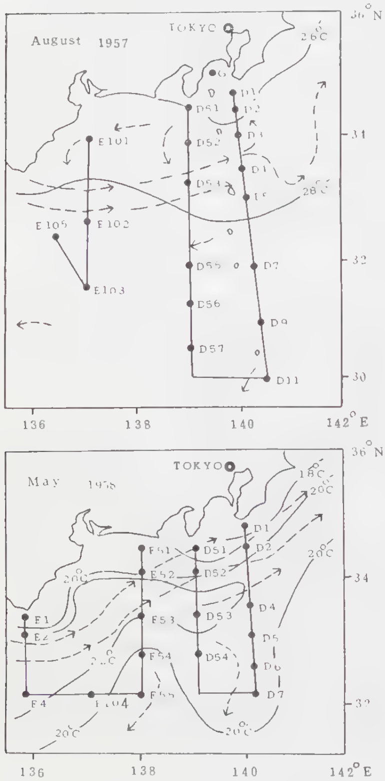

Fig. 1. Research area of the Kuroshio off the midcoast of Japan. The direction of the Kuroshio current is shown by broken line and the isotherm of surface water is shown by solid line.

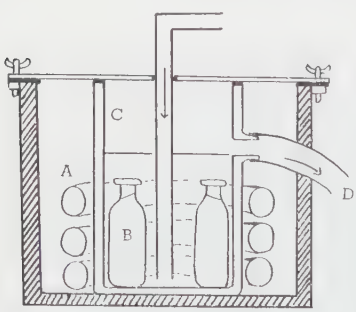

Fig. 2. Assimilation box. A: circline fluorescent lamp. B: assimilation bottle. C: glass incubator. D: water flowing.

As to light condition, the daily change in the illumination was observed with a photoelectric cell and simultaneously the solar radiation was measured with a Robitsch-pyrheliometer on the deck. The measurement of the submarine light intensity was carried out with a submarine photometer of selenium photocell and mainly the white light was measured during each cruise but at some stations the blue, green and red light were determined separately. Where direct photometry could not be employed due to unfavorable weather or limitation of time, submarine light intensities were estimated from transparency using formula $\alpha=1.7/d$ (cf. Ryther[9]), α being extinction coefficient of sea water and d being Secchi disc reading. This empirical formula was approximately confirmed by the data obtained during the present cruises.

As the measure of standing crop of phytoplankton, chlorophyll concentration in phytoplankton was used. The sample waters used for chlorophyll determination were taken with 10 l. bottles from depths of 0, 20, and 50 meters and stored in carboys. Sometimes the water was also collected from 10, 30, 100 and 200 meters. Normally 20 l. or 40 l. sample water was filtered through four sheets of filter paper (Toyo No. 101; 5 cm. in diameter) with a filtration apparatus. After the filter paper was dipped into the boiled water for about 30 seconds and dried in the air. The samples were kept in a desicator during the cruise. The chlorophyll concentration was determined in the authors' laboratory after the method described in a previous paper[10]. Photosynthetic rate was measured by C^{14} method. Briefly stated, the sample sea water was filled in two transparent and two darkened bottles and then 1 ml. of radioactive sodium carbonate solution containing 4–8 μc of C^{14} was added to each

Table 1. Chlorophyll content in the Kuroshio area off the southern midcoast of Japan. Chlorophyll amount is expressed in mg. per cubic meter of sea water.

August, 1957

Station	G	E101	E102	E103	E105	D51	D52	D53	D55
Date	1	2	2	3	3	15	15	15	16
Time	1940	1030	2130	0505	1235	1430	1810	2315	0920
Trans. m.	8	24	—	29	30	12	20	—	36
Depth in m.									
0	1.8	0.22	0.04	0.09	0.08	0.39	0.46	0.13	0.12
10	1.1	0.21	—	—	—	0.75	—	—	—
20	3.7	0.14	0.12	0.08	0.11	0.78	0.49	0.40	0.14
50	0.75	0.19	0.03	0.24	0.10	—	0.43	0.49	0.19
100				0.89	0.10	—	—	0.24	0.14

Station	D56	D57	D11	D9	D7	D5	D4	D2	D1
Date	16	16	17	17	17	18	18	18	18
Time	1400	1935	0910	1607	2256	0610	0935	1710	1930
Trans. m.	29	—	33	34	—	27	27	25	18
Depth in m.									
0	—	0.23	0.21	0.11	0.16	0.19	0.22	0.16	0.60
20	0.13	0.09	0.21	0.12	0.11	0.11	—	0.23	0.66
50	—	0.13	0.16	0.18	—	0.28	—	0.55	0.14
100	0.11	—	0.04	—	—	0.26	—	—	—

May, 1958

Station	D1	D2	D4	D5	D6	D7	D54	D53	D52
Date	1	1	2	2	2	2	3	3	3
Time	1730	2158	0655	1040	1528	1910	0650	1145	1655
Trans. m.	9	—	20	18	20	—	23	21	15
Depth in m.									
0	2.1	0.95	0.33	0.48	0.34	0.42	0.36	0.26	1.3
20	—	—	0.41	0.45	—	0.30	0.21	0.20	—
50	—	—	0.42	0.55	—	0.32	0.19	—	—
100	—	—	—	—	—	0.30	—	—	—

Station	D51	E51	E52	E54	E55	E104	E4	E2	E1
Date	7	7	7	8	8	8	8	9	9
Time	2015	1605	1955	0605	1050	1605	2350	0820	1140
Trans. m.	—	12	—	24	20	25	—	—	16
Depth in m.									
0	0.67	1.5	0.40	0.28	0.26	0.28	0.56	0.60	1.2
20	—	1.6	—	—	0.63	—	—	0.56	—
50	—	0.5	—	—	0.63	—	—	0.64	—

bottle. After 3 to 6 hours incubation, the phytoplankton was filtered with a membrane filter (No. 1), then washed and dried and its radioactivity was measured with a G. M. counter. The CO_2 assimilation was calculated according to Steeman Nielsen's method except that the dark fixation of C^{14} was subtracted from the value obtained in the light. For the incubation, an assimilation box was employed in this study. As shown in Fig. 2, this equipment is similar in principle to the Doty's[4)] but it is more simplified. Three circline fluorescent lamps are installed in a box, surrounding a glass incubator. Ten assimilation bottles can be set up in an incubator and each bottle is illuminated approximately at 15 klux. The bottles are always cooled with running water. The data of photosynthesis measured in the first cruise had to be omitted, because the abnormally large dark fixation was obtained by the contamination of stock solution in the amploules.

Results and Discussion

1. *Chlorophyll content in the Kuroshio area*

The chlorophyll amounts measured in the Kuroshio area are summarized in Table 1 and some other results obtained in the coastal water are also added in Table 2 as

Table 2. Chlorophyll content in some littoral waters.

Station	Tokyo Bay off Haneda	Tokyo Bay center	Tokyo Bay off Kisaratsu	Tokyo Bay Haneda	Shimoda Bay	Shimoda Bay
Date	May 20, '57	May 20,' 57	May 20, '57	Aug. 1, '57	June 12, '57	July 17, '57
Time	1130	1220	1330	1200	1100	1230
Trans. m.	6	7	5	8	—	14
Depth in m.						
0	2.7	1.6	1.8	1.8	0.6	1.0
5	4.9	2.1	1.8	—	—	—
10	3.2	2.9	1.8	1.1	0.5	1.6
20	2.7	3.0	2.2	3.7	0.6	1.2
50	—	—	—	0.8	1.1	0.8

a reference. Generally, the difference between the chlorophyll amount contained in the upper and deeper layer of the euphotic zone was slight in the Kuroshio area. Therefore, the stratification of chlorophyll distribution was not so remarkable as seen in the lake, where marked stratification can be observed in the stagnation period and sometimes even in the circulating period. In Fig. 3 is illustrated the horizontal distribution of chlorophyll in the cruising area, in which the concentration is expressed as mean value in the photosynthetic layer. From these results it may be concluded that the chlorophyll amount is high in the littoral water and generally decreases with increasing distance from the coast. In the pelagic area, the chorophyll is on the whole distributed homogeneously except for the areas near the islands and the shallow waters. In May the chlorophyll content showed roughly from 0.23–0.56

mg./m³. in the pelagic area and 0.60 to 2.1 mg./m³. in the littoral region. In August they decreased and varied from 0.08 to 0.2 mg./m³. in the former area and 0.37 to 2 mg./m³ in the latter. These results also showed good agreement with the seasonal variation of the zooplankton population in the same area investigated by Marumo[1),12)], who found that the weight of the zooplankton obtained by the net method indicated the maximum in May and minimum in August through three years. From the features of seasonal fluctuation of zooplankton, it may be considered that the range of the seasonal variation of chlorophyll content would also be limited within the value mentioned above.

The chlorophyll amount in the Kuroshio area accords fairly well with the values obtained by Doty and Oguri[3)], and Shimada[13)] in the pelagic Pacific waters, and Marshall[11)], and Ryther and Yentsch[15)] in the coastal waters.

If these values are compared with those of Japanese lakes, the coastal waters resemble the mesotrophic lakes and the pelagic waters are substantially the same with the oligotrophic lakes.

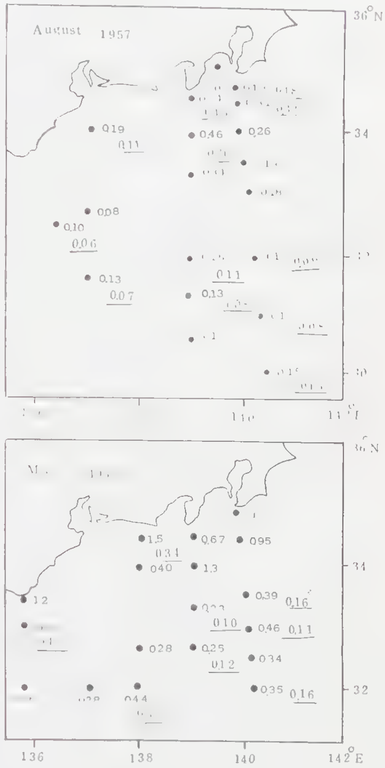

Fig. 3. Chlorophyll content and primary production of the Kuroshio area off the southern mid-coast of Japan. Small figures show the mean value of chlorophyll amount in the euphotic layer and large figures with the underline express the daily primary production in mg. C per unit surface (m²) of sea.

2. Relation of chlorophyll content to transparency and water temperature

The relationship between the transparency and the average value of chlorophyll content in the euphotic layer is shown in Fig. 4. A good linear reation can be seen between them in two different seasons and its correlation is closer than that obtained in Japanese lakes by the senior author[16)]. This good correlation probably depends upon the character of seston. Namely, the component of seston in the ocean mainly consists of plankton itself and the matter is displaced from that. This definite correlation may also suggest that the proportion of chlorophyll concentration to the seston and other organic matter dissolved in the water has been constant at least during these seasons. From these results it may be said that the chlorophyll content, in other words, the standing crop of phytoplankton can be estimated from the transparency, though more comprehensive data are naturally need. Indeed, for the purpose of converting the chlorophyll concentration into the

organic matter of phytoplankton, we must make adequate allowances for the difference in chlorophyll content among different phytoplankters.

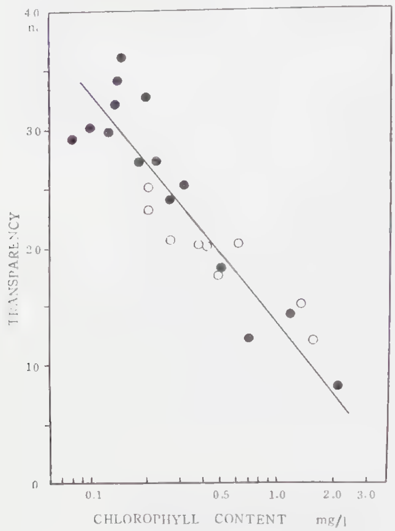

Fig. 4. Relationship between the transparency and chlorophyll content in the Kuroshio area. Chlorophyll amount is expressed as mean value in photosynthetic layer.
(●=August, 1957, ○=May, 1958)

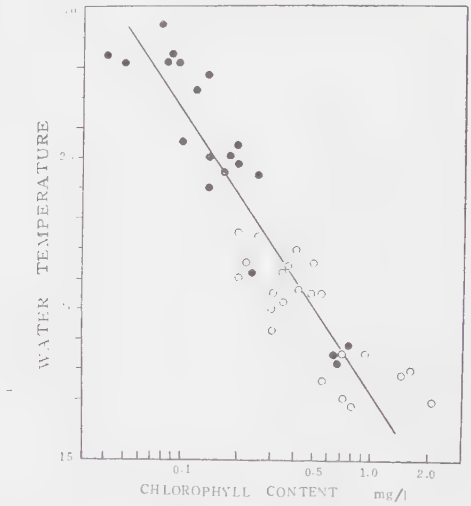

Fig. 5. Relationship between the water temperature and chlorophyll content in the Kuroshio area. Chlorophyll amount in figure is measured in sample water taken from the depth at which the relative light intensity reduces to 60–40% of the surface light intensity.
(●=August, 1957, ○=May, 1958)

Incidentally, the relation between the chlorophyll amount and *in situ* water temperature is plotted in Fig. 5. As seen in the figures, the high concentration of chlorophyll is rather found in the water of low temperature and it generally decreases with increasing water temperature. By the insufficiency of the determination concerning the nutrient elements in the water, precise explanation on this problem is so far difficult. But the feature of relation between the chlorophyll content and the temperature agrees fairly well with that of temperature assimilation curve obtained in fresh water diatom under opitimal light condition[17]. Consequently, it may be inferred that the definite correlation between the chlorophyll concentration and the water temperature is partly referred to the difference of productivity resulting from the geographical change in water temperature. On the other hand, it may be supposed that the turnover of plankton in high temperature water is more rapid than in the low.

3. *Photosynthetic rate of phytoplankton*

The photosynthetic rates of sample water taken from several stations are summarized in Table 3. The photosynthesis is measured by the C^{14} method at the optimum light intensity (15 klux) and *in situ* water temperature. Only, the results

Table 3. Productivity of sea water and photosynthetic rate of phytoplankton measured by C^{14} method.

Stations	Date and hour	Depth of sampling in m.	Water temp. *in situ*, °C	Experiment water temp. °C	Productivity	
					Cmg./m.³/hr.	Cmg./mg. chl./hr.
D5	May, 2	0	20.5	25	0.62	1.3
	0930	20	20.5	〃	0.41	0.91
		50	20.5	〃	0.36	0.63
D7	May, 2	20	20.5	25	0.20	0.67
	1810	50	20.1	〃	0.30	1.00
D54	May, 3	0	21.4	25	0.29	0.66
	0600	20	21.6	〃	0.07	0.33
		50	19.2	〃	0.10	0.53
D52	May, 3 1615	0	19.2	23	2.2	1.7
E51	May, 7	0	19.2	21	2.4	1.6
	1530	20	18.4	〃	1.7	1.1
		50	18.1	〃	0.77	1.5
E55	May, 8	0	18.6	21	0.66	2.5
	0955	20	18.6	〃	1.5	2.4
		50	18.5	〃	1.2	1.8
E2	May, 9	0	22.6	23	0.82	1.4
	0720	20	22.8	〃	0.69	1.3
E1	May, 9 1115	0	19.2	21	4.1	3.4
29°56′N	July, 27	0	26.5	26	0.20	1.1
145°00′E	2130	20	25.1	〃	0.24	1.7
		50	22.3	〃	0.08	0.45
32°00′N	July, 28	0	26.3	26	0.24	1.3
145°00′E	1455	20	25.1	〃	0.10	1.0
		50	21.7	〃	0.08	0.5
34°01′N	July, 29	0	25.8	25	1.32	—
144°56′N	0858	20	22.7	〃	0.44	2.9
		50	22.1	〃	0.73	2.2

at the stations D4, D5 and D7 were measured at 25° which is slightly higher than that *in situ* water temperature (20°–21°), therefore these values seem to be a little reduced from the values *in situ*. It can be seen from these figures that the productivity of water in the littoral region is about 1 to 4 mg. C/mg. chl./hr., and 0.1 to 0.6 mg. C/mg. chl./hr., in the pelagic area. If we consider the photosynthetic rate per unit amount of chlorophyll, these values correspond to 1 to 3 mg. C/mg. chl./hr. in the former area and 0.3 to 1.7 mg. C/mg. chl./hr. in the latter, respectively. The photosynthetic rate measured in northern area of the Kuroshio current in summer 1958 was about 0.1–0.2 mg. C/m³./hr. or 0.5–0.7 mg. C/mg. chl./hr.. These values clearly showed a good agreement with the above ones. However, the values of photosynthetic rates measured in the Kuroshio area are generally a little lower than the values

2.5–7.3 mg. C/mg. chl./hr., which were reported by Holmes *et al.*[6], in the tropical Eastern Pacific Ocean. The causes of this discrepancy seem to arise from the differences of the water temperatures and nutrient conditions, etc.

The phytoplankton distributed in the euphotic layer showed nearly similar potential photosynthetic activity but sometimes low photosynthetic rate was measured with the sample which was taken from the layer near the compensation depth. The low photosynthetic activity may be attributable to the characteristic shade type of photosynthesis as affected by phytoplankton living in deeper layer. The same phenomenon had also been found by the senior author in the lakes[16].

4. Primary production

The primary production of water has generally been measured by three methods as follows; *in situ*, tank and chlorophyll methods (cf. Steemann Nielsen,[2] Ryther[18]). The "*in situ*" method could not be used during the cruises, hence the production was mainly determined by the latter two methods. The daily amount of photosynthesis taking place in various layers would be possible to estimate from the combination of the daily incident surface radiation, relative submarine illumination and light-photosynthesis curve of sample water taken from each depth. Then the daily primary production beneath a square meter of sea surface may be calculated by integration of the values so obtained for each depth. On board the ship, however, it was a very difficult work trying to make such a light-photosynthesis curve at each station. According to Ryther[19], if one knows the general photosynthesis light curve for marine phytoplankton and the photosynthetic rate in sample water at opitimum light intensity, it is possible to obtain from these data the relation between phytosynthesis and light intensity in sample water. Employing this method, the calculation of production was performed. The general photosynthesis-light curve was constructed from the experiments made at the Shimoda Marine Biological Institute, Tokyo University of Education. The results of calculation are shown in Fig. 3. But the "tank" method was used at some stations and the daily production was determined for other stations by the chlorophyll method. This method was only employed in the ocean by Ryther and Yentsch[9],[15], though it had already been introduced in the study of primary production of the lake by several investigators as Manning and Juday[20], Hogetsu and Ichimura[10], Saijo[21] etc. The chlorophyll method is similar in principle to the tank method except that the photosynthetic rate is indicated by unit amount of chlorophyll. The daily rate of primary production is calculated from the vertical distribution of chlorophyll, relative submarine illumination and incident radiation, using a general photosynthesis-light curve and the value of photosynthesis per mg. chlorophyll at optimum light intensity. The general photosynthesis-light curve was taken from Ryther's figure (1956, p. 65, Fig. 2) and the mean values at light optimum actually used in this calculation were 1.5 mg. C/mg. chl./hr. in the pelagic water and 3 mg. C/mg. chl./hr. in the coastal water.

These photosynthetic rates were little lower than 3.7 mg. C/mg. chl./hr., which was used by Ryther in his calculation. The results of this calculation are given in Fig. 3. The productivity of the Kuroshio area approximately coincides with the values measured by Steemann Nielsen[2] in the pelagic water of the Pacific near Hawaiian island and of the middle Atlantic. It should be noted the difference of the production between August and May is not so much distinguished in the value per unit square of sea surface as in the chlorophyll content. This may be easily interpreted when the difference of the transparency has been taken into consideration. In August the depth of transparency reaches 30 m. or more whereby the depth of euphotic zone becomes quite large, while contrarily in May the depth of euphotic zone decreases with the decreasing of transparency. Namely, the organic matter production in a phytoplankton community is determined with the productive structure of the community and the productivity of algae. Such contradiction between the density and productivity of phytoplankton community may be given as one of the most important problems on the plant ecology. Assuming the seasonal variation in the Kuroshio area being rather small throughout the year, the annual production is comparable with the production of the Sargasso[22] and on the Fladen ground of the northern North Sea[23].

Summary

The primary production in the Kuroshio off the southern midcoast of Japan was measured during the cruises in August 1957 and in May 1958. The chlorophyll amount in the sea water was high in the littoral region and it generally decreased with increasing distance from the coast. The mean value of chlorophyll content in euphotic zone showed roughly from 0.23 to 2.2 mg./m.^{3} in May and 0.1 to 2.0 mg./m.^{3} in August. A good linear relationship can be seen between the transparency and the mean value of chlorophyll content in the euphotic zone and this suggests that the standing crop of phytoplankton can be estimated from the depth of transparency. The definite correlation was also found between the chlorophyll content and the water temperature at least during these two months.

The photosynthetic rate was measured by the C^{14} method at opitmum light intensity (15 klux) and *in situ* water temperature. The productivity of water in the littoral region was about 1 to 4 mg. C/m.^{3}/hr., and 0.1 to 0.6 mg. C/m.^{3}/hr. in the pelagic area. These values correspond to 1 to 3 mg. C/mg. chl./hr., and 0.3 to 1.7 mg. C/mg. chl./hr., respectively. Based on these values, the primary production was determined by the tank and chlorophyll methods. Daily production ranged in the pelagic area from 70 to 150 mg. C/m.^{2}/day and from 300 to 500 mg. C/m.^{2}/day in the coastal region. It may be inferred from these results that the Kuroshio off the southern midcoast of Japan belongs to one of the low productive area of the ocean.

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摘　　要

海洋の基礎生産に関する研究は最近いちじるしく発展が見られる。特に Steemann Nielsen による C^{14} の導入は外洋の基礎生産の測定も容易ならしめた。しかるに西北太平洋については，わずかに二三の研究を見るにすぎない。著者等は黒潮ならびに親潮の基礎生産の測定を行なったので，ここにその一部である本邦南方黒潮海域（図 1）の 1957 年 8 月，1958 年 5 月の測定結果について報告する。

植物プランクトンの現存量としてクロロフィルの定量を行なった。沿岸部で 5 月 0.6-2.1 mg./m.³, 8 月 0.4-2.0 mg./m.³, 外洋では 5 月 0.23-0.56 mg./m.³, 8 月 0.08-0.2 mg./m.³ であった（図 3）明らかにいわゆる island mass effect の現象が見られる。クロロフィルの垂直分布は湖沼程には明瞭な成層は認められなかった（表 1-2)。海域の透明度とクロロフィル含量との間には直線的相関が得られた(図 4)。

これは海洋ではプランクトンガ水中光度を弱める主要要因となり，湖沼よりも植物プランクトンのエネルギー効率の大きいことを示している。

植物プランクトンの生産力測定には C^{14} を用いた。光飽和における生産力は沿岸部で 1-4 mg. C/m.³/hr. 外洋で 0.1-0.6 mg. C/m.³/hr. を示した。一日の生産量を tank 法および chlorophyll 法で求めた結果，沿岸部で 0.3-0.5 g. C/m.²/day，外洋で 0.07-0.15 g. C/m.²/day を得た。この値は大西洋の Sargasso や北海の Fladen 海域で報告されている値に近い。著者等によって調査された黒潮海域は他の海域に比して基礎生産力はかなり低いと考えられる。

Mechanism of Growth Promotion by Ferrous Sulfate.

by Hiroh SHIBAOKA* and Toshio YAMAKI*

柴岡弘郎*・八巻敏雄*： 鉄イオンによる生長促進の機構について

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Introduction

Previously, the authors demonstrated the promoting effects of ferrous sulfate on the auxin-induced curvature of *Avena* coleoptiles, and showed that the application of this phenomenon could make the ordinary *Avena* test more sensitive[1].

Further investigations on the mechanism of this promotive action of ferrous ions on the *Avena* curvature test may be necessary not only to understand the results obtained by means of the Fe^{++}-applied sensitive *Avena* curvature test, but also to clarify the mechanism of the growth of *Avena* coleoptiles induced by auxin.

Material and Methods

Avena Straight Growth Test: *Avena* seedlings (Victory No. 1) were grown at 25° in darkness, and when the height of the seedlings reached 2.5 cm., the tips of the coleoptiles, 1 mm. in length, were removed. Three hours after this treatment, the further 4 mm. were cut off and the next 5 mm. sections were used for this experiment. Twenty sections were floated on 10 ml. of test solution, and their lengths were measured after 18 hour incubation at 20° in darkness. All test solutions contained 2% sucrose and were adjusted to pH 5.0 at the beginning of the experiment.

Avena Curvature Test: Using similar materials to those of the straight growth test, the curvature test was performed in standard manner as described by Thimann[2].

IAA-Oxidase Preparation: A modified method of that employed by Tang *et al*[3] for the preparation of the IAA-oxidase of *Avena* coleoptiles was used, and *Avena* seedlings grown at 25° in darkness for four days being used as the plant material. At this stage the coleoptile has not yet been broken through by the primary leaf. The primary leaves were pulled out from the harvested coleoptiles and about 20 g. in fresh weight of whole coleoptiles were ground with 80 ml. of cold water in a chilled Waring blendor for 2 minutes. The homogenate was centrifuged for 15 minutes at 3600 *g* and the supernatant was decanted, added to an equal volume of cold acetone, kept at 0° for 30 minutes and centrifuged at 3000 *g* for 15 minutes.

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The supernatant was discarded and the precipitate was suspended in 10 ml. of cold water, left overnight at 0° and recentrifuged at 3600 g for 10 minutes. The resulting clear supernatant was used as the enzyme solution.

Estimation of IAA-Oxidase Activity: One ml. of the enzyme solution, 0.5ml. of M/20 phosphate buffer containing 60 μg. of IAA and 0.5 ml. of $FeSO_4$ solution in various concentrations were mixed and the mixture was incubated at 30° by shaking. After one hour incubation the reaction mixture was adjusted to pH 3.0 with diluted H_2SO_4 and IAA was extracted with ether from the acidified mixture. The extraction was repeated 3 times and the ether extracts were gathered, evaporated to dryness, dissolved into 1 ml. of distilled water and added to 4 ml. of Salkowski-Tang's reagent (0.5 M $FeCl_3$-H_2O-H_2SO_4, 15 : 500 : 300 v/v)[4]. After 30 minutes at room temperature, the color density was measured using a photoelectric photometer with a green filter, and the quantity of IAA which disappeared from the reaction mixture during the incubation period was estimated.

Estimation of Auxin Transport: Coleoptiles similar to those used in the *Avena* straight growth test were prepared. Three hours after decapitation, the topmost 4mm. was removed and the following 3.2 mm. cylinder was used. The primary leaf filling the coleoptile clinder was pulled out. The cylinder was placed vertically on a pure agar block (2%, $2\times2\times2$ mm.^{3}), and on the upper cut surface of the cylinder was placed an agar block (2%, $2\times2\times2$ mm.^{3}) which had been soaked in the test solution for 3 hours. Taking necessary precautions against desiccation, the coleoptile cylinder with the agar blocks was kept at 25° in darkness. After a fixed period, the auxin activity in the lower block was analysed by the *Avena* curvature test. Before the curvature test, a small amount of 10^{-2}M $FeSO_4$ solution was added to the agar blocks to be tested. The addition of 10^{-2}M $FeSO_4$ solution brings the concentration of $FeSO_4$ in the agar block to its optimum concentration or thereabout, and therefore it is possible to disregard the effect of $FeSO_4$ given in the upper agar block, even if such $FeSO_4$ could be transported into the lower block. Because, at the optimum concentrations of ferrous ions, the small change in the quantity of the ions does not affect their promoting effect on the growth induced by auxin[1].

Results

(1) Effect of $FeSO_4$ on *Avena* straight growth test.

Fig. 1 shows the effect of $FeSO_4$ on the elongation of *Avena* coleoptile sections and the quantity of IAA remaining in each test solution after the incubation period. The initial concentration of IAA in the test solution was 0.05 mg./l.. The estimation of the quantity of the remaining IAA was performed as follows: After incubation period, 10 ml. of the test solution was added to the same volume of 2×10^{-2}M $FeSO_4$ solution, and 12 pieces of pure agar blocks (2%, $2\times2\times2$ mm.^{3}) were soaked into this mixture for 3 hours and these agar blocks were analysed by the *Avena* curvature test. As the control of this experiment, the test solution having no coleoptile sections

was incubated for 3 hours and the quantity of IAA in it was estimated by the same procedure.

As shown in Fig. 1, even at the low concentration of 1/500 of the optimum concentration for the *Avena* curvature, i. e. at 10^{-5}M, $FeSO_4$ still promotes the elongation of *Avena* coleoptile sections induced by IAA. It is also shown that the quantity of IAA which disappeared from the test solution which contained $FeSO_4$ at the

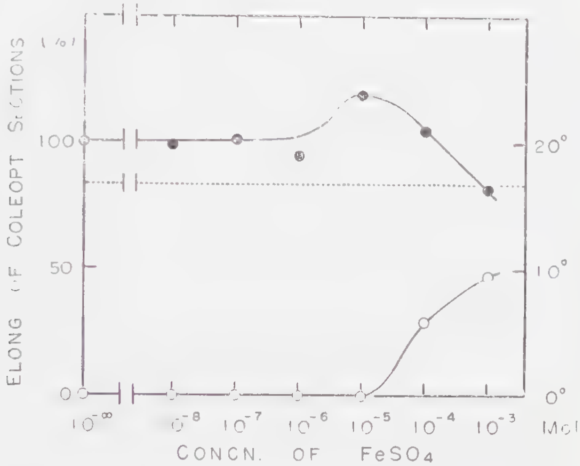

Fig. 1. Elongation of *Avena* coleoptile sections in 2% sucrose solution with 0.05 mg./l. of IAA and various amounts of $FeSO_4$. Growth was measured after 18 hours at 25°: in darkness, and expressed as percentage of that of the Fe^{++}-free control. The amount of IAA in the test solution was estimated after 18 hour incubation. Quantity of IAA was measured by *Avena* curvature test.

—●— elongation of sections
—○— remaining IAA
------ remaining IAA in control (no sections)

concentration of 10^{-4}M is smaller than that which disappeared from the test solution containing no $FeSO_4$, while these two test solutions induce almost the same elongation of the coleoptile sections. These results may indicate that some amounts of the IAA in the test solution are consumed by the sections during the incubation period without contribution to the growth of the sections, and this fact makes us wonder whether the addition of $FeSO_4$ suppressed such consumption of IAA. In the case that the test solution has no or a small amount of $FeSO_4$ ($10^{-\infty}\sim10^{-5}$M), the IAA existing in the test solution at the beginning of the test disappeared completely during the 18 hour incubation. This fact points to the supposition that the infiltrating velocity of IAA into the plant tissues may not be the limiting factor of the growth of *Avena* coleoptile sections.

Fig. 2 shows the effect of $FeSO_4$ on the elongation of *Avena* coleoptile sections floated on NAA solution. NAA was used at the concentration of 0.3 mg./l.. It is

shown that ferrous ions are not promotive, but rather inhibitory on the elongation of *Avena* coleoptile sections induced by NAA. This result probably denies the possibility that the promoting effect of $FeSO_4$ on the elongation of *Avena* coleoptile

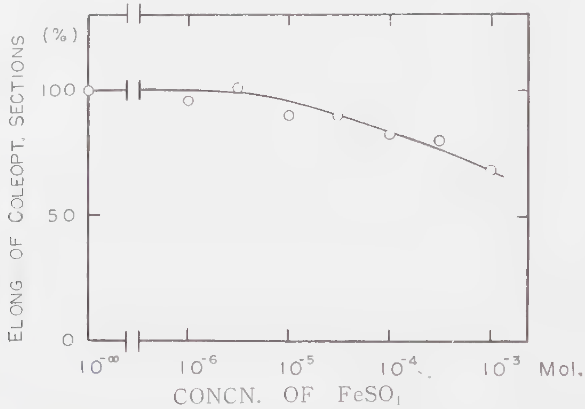

Fig. 2. Elongation of *Avena* coleoptile sections in 2% sucrose solution with 0.3 mg./l. of NAA and various amounts of $FeSO_4$. Growth was measured after 18 hours at 25°, and expressed as percentage of that of the Fe^{++}-free control.

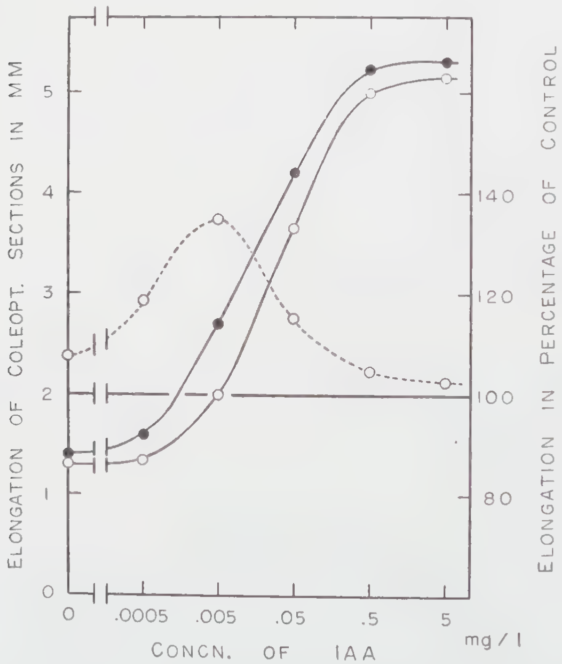

Fig. 3. Effect of $FeSO_4$ on elongation of *Avena* coleoptile sections in 2% sucrose solution with various concentrations of IAA. Growth was measured after 18 hours at 25°.

—●— with 10^{-5}M $FeSO_4$
—○— without $FeSO_4$
------ promotion rate in percentage

sections may be due to the increasing of the infiltrating velocity of auxin or some substances having physiological activities similar to those of auxin. And this result may also deny that $FeSO_4$ promotes the physiological or biochemical steps in the process of the growth which are affected equally by NAA and IAA. So, in case of the section growth, $FeSO_4$ can be supposed to act specifically on the action of IAA. From this supposition and the fact that IAA-oxidase does not destroy NAA, it may be natural to support the idea that the growth promoting effect of $FeSO_4$ is due to its inactivating action on IAA-oxidase.

Fig. 3 shows the elongation of coleoptile sections in test solutions which contain $FeSO_4$ at 10^{-5}M, the optimum concentration, and IAA of various concentrations. As shown in Fig. 3, when IAA concentration is low, the promotive effect of $FeSO_4$ is remarkable, and when IAA is used in high concentration, the effect is slight. These results can be explained fairly well, if $FeSO_4$ acts inhibitorily on the destruction of IAA by coleoptile sections. That is, under high concentration of IAA, even if sections destroy IAA in the test solution, the quantity of destroyed IAA is so small in comparison with the quantity of remaining IAA, that such destruction can affect very little the growth promoting action of IAA in the test solution. Consequently, in the case of high concentration of IAA, the addition of ferrous ions that seem to inhibit the destruction of IAA must have no relation to the section growth. And when the amount of added IAA is small, the concentration of IAA in the test solution can be changed by the destruction of IAA, so the addition of $FeSO_4$ that seems to inhibit such destruction is able to have close connection with the section growth.

The same experiment was made using NAA as auxin. Result is shown in Table 1. At the concentration of 10^{-5}M, $FeSO_4$ does not show any promotive effect on the elongation of sections in every concentration of NAA.

Table 1

Effect of $FeSO_4$ on elongation of *Avena* coleoptile sections in 2% sucrose solution with various concentrations of NAA. $FeSO_4$ was used at 10^{-5}M.

Concn. of NAA (mg./l.)	$\frac{\text{Growth in presence of } Fe^{++}}{\text{Growth in absence of } Fe^{++}} \times 100$
0	107.5
0.005	91.0
0·05	91.0
0.5	95.0
5.0	95.0

As already described, $FeSO_4$ causes the most obvious promotive effect on IAA action when the concentrations of $FeSO_4$ and IAA are 10^{-5}M and 0.005 mg./l. respectively. Using such concentrations of IAA and $FeSO_4$, the change of the rate

of growth promotion by $FeSO_4$ according to time was observed. The result is shown in Fig. 4: the effect of $FeSO_4$ cannot be found at the start, but it appeared and increased gradually.

This fact may be explained as follows: At the start, the quantity of IAA in each test solution is the same, irrespective of the presence or absence of ferrous ions, so the sections in each test solution elongate equally. As time goes on, however, a larger amount of IAA disappears from the test solution having no ferrous ion than from that containing ferrous ions, so the elongation of sections in each test solution becomes different, i. e. the promoting effect of ferrous ions appears gradually.

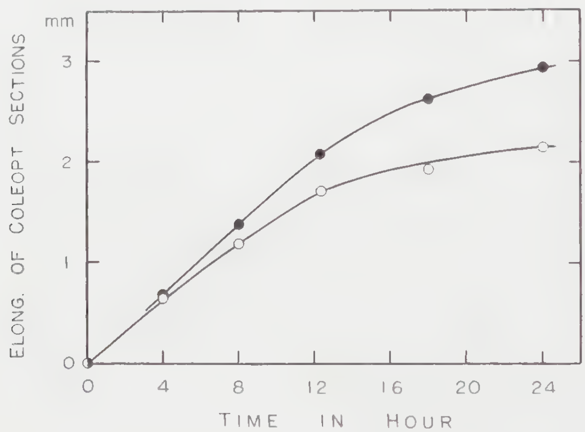

Fig. 4. Effect of $FeSO_4$ on course of elongation of *Avena* coleoptile sections in 2% sucrose solution with 0.005 mg./l. of IAA.
—●— with 10^{-5}M $FeSO_4$
—○— without $FeSO_4$

If ferrous ions directly promote the activity of auxin, the promotion must appear from the beginning. The experimental result, however, did not support this assumption.

(2) Effect of $FeSO_4$ on enzymatic destruction of IAA.

According to the above described results, it may be concluded that the most probable mode of action of ferrous ions is that they seem to inhibit the action of an enzyme which destroys IAA. Moreover, the inhibitory effect of ferrous ions on IAA-oxidase activity has been reported by several workers, namely Wagenknecht *et al*[5], who found the inhibition of 2×10^{-3}M ferrous citrate on the IAA-oxidase of bean roots, Galston *et al*[6], who used ferrous sulfate at 5×10^{-4}M and found its inhibitory effect on the oxidase obtained from pea epicotyls, and Murakami *et al*[7], who observed the inhibitory effect of 8×10^{-4}M ferrous sulfate on the oxidase of rice plants. Therefore, our experiment has been made to ascertain:whether or not ferrous sulfate, at the effective concentration for the straight growth test, inhibits the activity of the IAA-oxidase obtained from *Avena* coleoptiles. The experiment was carried out at pH 6.2.

Table 2

Effect of pH on IAA-oxidase obtained from *Avena* coleoptiles. Reaction mixture was composed of 1 ml. of the enzyme solution, 0.5 ml. of M/20 phosphate buffer with 60 μg. of IAA and 0.5 ml. H_2O. The mixture was incubated for 60 minutes at 30°.

pH	Remaining IAA (μg.)	Destroyed IAA (μg.)
1.9	60.0	0.0
5.5	59.5	0.5
6.2	42.0	18.0
6.6	34.0	26.0

At this pH, the activity of IAA-oxidase is considerably high (shown in Table 2), and ferrous ions are barely stable. Table 3 shows the obvious inhibiting effect of ferrous ions on the IAA-oxidase activity. This result may support the assumption that the growth promoting action of ferrous ions derives from their inhibiting effect on IAA-oxidase.

Table 3

Effect of $FeSO_4$ on IAA-oxidase from *Avena* coleoptiles. Reaction mixture was composed of 1 ml. of the enzyme solution, 0.5 ml. of M/20 phosphate buffer with 60 μg. of IAA and 0.5 ml. of $FeSO_4$ solution. The mixture was incubated for 30 minutes at 30°, pH 6.2.

Concn. of $FeSO_4$ (M)	Remaining IAA (μg.)	Destroyed IAA (μg.)
$10^{-\infty}$	42.0	18.0
10^{-5}	52.0	8.0
10^{-4}	51.0	9.0
10^{-3}	55.0	5.0
control (no enzyme)	59.0	1.0

(3) Effect of $FeSO_4$ on the course of *Avena* coleoptile curvature.

Previously, the authors reported that ferrous ions promote the *Avena* coleoptile curvature induced by NAA as well as that induced by IAA[1]. The present experiment, however, shows that this metallic ion does not promote the elongation of coleoptile sections induced by NAA, while it increases the elongation caused by IAA. These facts bring to the authors the idea that ferrous sulfate promotes two kinds of *Avena* tests, i. e. straight growth test and curvature test, in two different manners. So, the further investigation on the mechanism of promoting effect of $FeSO_4$ on the *Avena* curvature test was made.

Fig. 5 shows the course of *Avena* coleoptile curvature after application of the agar block. The test solution in which the agar blocks were soaked contained 0.050

or 0.025 mg./l. of IAA and $10^{-\infty}$ or 5×10^{-3}M of $FeSO_4$. As shown in Fig. 5, the promoting effect of $FeSO_4$ was highest soon after the application of the agar block and decreased gradually. A similar result was obtained using NAA; it is shown in

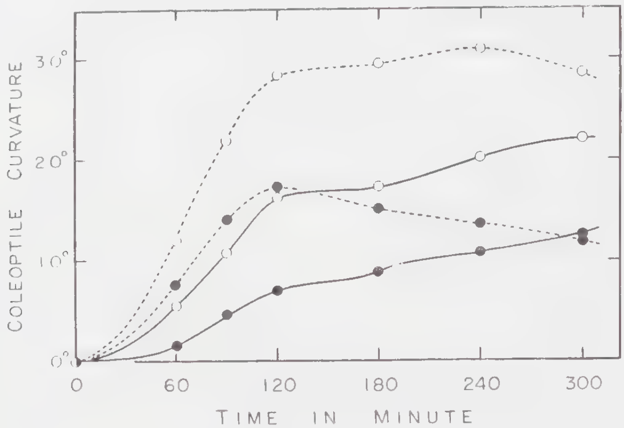

Fig. 5. Effect of $FeSO_4$ on *Avena* coleoptile curvature according to time.

—●— 0.025 mg./l. IAA
—○— 0.050 mg./l. IAA
-●- 0.025 mg./l. IAA $+5 \times 10^{-3}$M $FeSO_4$
-○- 0.050 mg./l. IAA $+5 \times 10^{-3}$M $FeSO_4$

Table 4. These results cannot be explained by the inhibiting action of $FeSO_4$ on IAA-oxidase, because NAA does not seem to be destroyed by IAA-oxidase. These results, however, are not inconsistent with the assumption that ferrous ions promote the infiltration of auxin from the agar blocks into the coleoptiles of *Avena* seedlings. Then, the following experiments were made to examine this possibility.

Table 4

Effect of $FeSO_4$ on *Avena* coleoptile curvature according to time.

Time in minute	Coleoptile curvature caused by: 0.3 mg./l. NAA	0.3 mg./l. NAA $+5 \times 10^{-3}$M $FeSO_4$
60	3.1°	9.3°
120	11.4°	20.5°
300	13.1°	14.5°

(4) Effect of $FeSO_4$ on the transport of auxin through *Avena* coleoptile section.

The test solution, in which were soaked the agar blocks to be placed on the upper cut surface of the coleoptile cylinder, contained 0.05 mg./l. of IAA or 1.0 mg./l. of NAA and $10^{-\infty}$ or 5×10^{-3}M of $FeSO_4$ and was adjusted to pH 5.0. The transport was observed by the method described in the preceding chapter. The time allowed

for the transport of IAA was 60 or 90 minutes, and that for NAA was 120 minutes. The results are summarized in Table 5. A larger amount of IAA was transported from the agar block containing $FeSO_4$ than from that having no $FeSO_4$. As a similar

Table 5

Effect of $FeSO_4$ on the transport of auxin through 3.2 mm. of coleoptile cylinder.

Composition of agar blocks applied at upper surface	Length of the diffusion period in min.	Amount of auxin in the agar blocks applied at lower surface*
0.05 mg./l. IAA	60	4.6°
〃 $+5\times10^{-3}$M $FeSO_4$	60	8.6°
0.05 mg./l. IAA	90	12.1°
〃 $+5\times10^{-3}$M $FeSO_4$	90	15.1°
1.0 mg./l. NAA	120	4.7°
〃 $+5\times10^{-3}$M $FeSO_4$	120	8.8°

* Expressed by degrees of *Avena* curvatures.

result was obtained by using NAA, it can be concluded that these results did not depend on the inhibiting effect of ferrous ions on the IAA-oxidase of *Avena* coleoptile cylinders, but were due to the promoting effect of the ions on the transport of auxin. And it may also be supposed that the promoting effect of ferrous ions on the *Avena* curvature test is due to their promoting effect on the transport or infiltration of auxins.

Discussion

As the possible modes of action of ferrous ions in the two kinds of *Avena* tests, i. e. the straight growth test and the curvature test, the following three cases can be supposed: (1) Ferrous ions promote the infiltration or transport of auxin into coleoptiles. (2) They increase the activity of auxin or the sensitivity of plant tissues to auxin. (3) They inhibit the action of an enzyme which destroys auxin. The following discussion will be made to examine the probability of these three modes of action.

The measurement of IAA remaining in the test solution, after the straight growth test, showed that, from the test solution containing no ferrous sulfate, the given IAA disappeared completely. This fact indicates that the possibility of (1) is little in case of the straight growth test. It was also shown that the remaining IAA was found in the test solution having ferrous sulfate at the concentration of 10^{-4}M, in which the coleoptile sections elongated as much as those in the ferrous ions free control solution. This fact gives us the suggestion that, from the test solution containing no ferrous ion, considerable amounts of IAA disappeared during the incubation period without relation to the growth of sections, and that ferrous

ions have the activity of preventing such disappearance. This suggestion may support the assumption (3). Ferrous sulfate was found to have no promotive effect on the elongation of sections induced by NAA. This result also indicates that the assumption (1) cannot be accepted. In general, IAA-oxidase is known to have no activity on the oxidation of NAA, so the above result may also support the possibility of (3). Thimann reported that Co^{++} promoted the growth of both *Avena* coleoptile sections and pea epicotyl sections in NAA solution as well as in IAA solution[8]. Thimann's report and the present result indicate that Fe^{++} and Co^{++} act on the growth through different ways of action. As far as our experiments are concerned, ferrous ions, did not show any promotive effect in high concentrations of IAA, while their effect appeared obviously in low concentrations of IAA. This observation may also support the assumption (3), because, if (3) is supposed to be right, it is possible to give the following explanation. In the case when high concentration of IAA is given in the test solution, the change of the IAA concentration resulting from the action of IAA-oxidase is too small to have any effect on the growth of sections in this test solution. So, the addition of an inhibitor of IAA-oxidase cannot show any effect on the section growth. But in case of the low concentration of IAA, the change of the IAA concentration by the oxidase is considerable, and an inhibitor of this enzyme promotes the section growth obviously. Furthermore, it is also inferred by this result that the action of Fe^{++} is not the same as that of Co^{++} or Mn^{++}, which shows its growth promoting effect in high concentrations of auxin[8),9)]. It is found that the promotive effect of ferrous ions on the elongation of *Avena* coleoptlile sections is not appreciable in the early period of the incubation. This result points to the improbability of the assumption (2), because, if (2) is the reason for the effect of ferrous ions, this effect must be found from the beginning of the incubation. Because all the results described above supported the assumption (3) as the most possible mechanism for the action of ferrous ions, the effect of ferrous sulfate on the destruction of IAA was examined *in vitro*. And the inhibiting effect of ferrous ions on the activity of IAA-oxidase was detected, at the concentration of Fe^{++} that was found to be effective on the section growth. On the basis of the already mentioned data, it may be concluded that the assumptions (1) and (2) cannot be the mechanisms for the promoting effect of ferrous ions on the elongation of *Avena* coleoptile sections, but that (3) might very possibly be the mechanism.

If ferrous ions act on the two different kinds of *Avena* tests through the same mechanism, it is impossible to explain why ferrous ions do not promote the elongation of the coleoptile sections in NAA, while they promote the coleoptile curvature induced by NAA. So, the mechanism of the promoting effect of ferrous ions on the *Avena* curvature test must be different from that of their promoting effect on the straight growth test. In case of the curvature test, the possibility of the assumption (1) is great, according to the following two results of our experiment. One is that the promotive effect of ferrous ions is obvious during the early stage of the curvature

test, then decreases gradually. The other is that a larger amount of IAA is transported through the given length of the coleoptile cylinder from the auxin-agar block containing ferrous sulfate than from that containing no ferrous sulfate.

As (1) seems to be a proper explanation for the promoting effect of ferrous ions on the coleoptile curvature, it seems natural to expect that ferrous ions promote the action of other auxins than the already tested IAA and NAA. Therefore, it is also natural to suppose that the application of ferrous ions to the *Avena* curvature test may be useful for the detection of the unkown growth substances.

The present paper has demonstrated that the promoting effect of ferrous ions on the curvature test may be due to their accelerating effect on the transport or infiltration of auxin from the agar block into the coleoptile, and that their effect on the straight growth test may be caused mainly by their inhibiting effect on IAA-oxidase. This paper, however, does not give any reason why ferrous ions promote the transport or infiltration of auxin and why they inhibit the action of IAA-oxidase, so the essential problems still remain unsolved. And, though it has not been clarified whether the mechanism (2) has any role in the effect of ferrous sulfate on the curvature test, further investigations on this point are also required.

Summary

(1) Ferrous sulfate, at the concentration of 10^{-5}M, promotes the elongation of *Avena* coleoptile sections in IAA solution, at the concentration of 0.05 mg./l..

(2) When the straight growth test is carried out, ferrous sulfate, at concentrations higher than 10^{-5}M, prevents the disappearance of IAA from the test solution during the incubation period.

(3) Ferrous sulfate does not promote the elongation of *Avena* coleoptile sections in NAA.

(4) The promotive action of ferrous sulfate on the elongation of the sections appears obviously when IAA is used at low concentrations and the incubation period is prolonged.

(5) Ferrous sulfate, at the concentration having promotive effect on the section growth, inhibits the action of IAA-oxidase obtained from *Avena* coleoptiles.

(6) The promoting effect of ferrous sulfate is larger in the earlier period of the *Avena* curvature test, and go decreasing thereafter.

(7) Ferrous sulfate promotes the transport of both IAA and NAA through a given length of the coleoptile section.

(8) Based on the data summarized above, it may be concluded that ferrous sulfate acts promotively on the *Avena* curvature test through its promoting effect on the transport or infiltration of auxin, and on the straight growth test through its inhibiting effect on IAA-oxidase.

(9) Therefore, the *Avena* curvature test sensitized by the application of ferrous ions seems to be useful for the detection of small amounts of auxin.

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摘　　要

前報において $FeSO_4$ がアベナ屈曲試験に対し促進的に働らくことを述べた。今回はこの鉄イオンの促進作用の機構を明らかにするために行なった実験についての報告を行なう。

$FeSO_4$ は 10^{-5}M で 0.05 mg./l. の IAA によってひきおこされるアベナ子葉鞘切片の伸長を増大させる。伸長試験後，検液中に残っている IAA をはかると $10^{-\infty}$〜10^{-5}M の $FeSO_4$ をふくむ検液からは完全に IAA が消失しているが，10^{-4}M 以上の $FeSO_4$ が加えられていた検液中には IAA が残っている。$FeSO_4$ は NAA による切片の伸長に対しては促進的でない。$FeSO_4$ の伸長促進作用は IAA 濃度の高い時にはほとんど現れず，IAA 濃度の低い時に明りょうに現れる。短時間の伸長試験ではこの促進作用は見られず，長時間の試験ではじめていちじるしく現れる。$FeSO_4$ は伸長促進作用を示す濃度 (10^{-5}M) でもアベナ子葉鞘からとった IAA 酸化酵素による IAA の破壊を阻害する。などが明らかにされ，これらの実験事実から，伸長試験に対する $FeSO_4$ の促進作用は，$FeSO_4$ がオーキシンの分解を抑制するため，という結論がひき出された。

また，屈曲試験については，$FeSO_4$ の IAA，または NAA によってひきおこされるアベナ子葉鞘の屈曲に対する促進作用は，短時間のテストでも明らかにみられ，$FeSO_4$ はまた，上下を切った子葉鞘円筒の上側の切口においた寒天片中の IAA，または NAA が円筒組織内を通過して円筒の下側の切口に附着させた寒天片中に移動するのを促進する。などが観察され，屈曲試験に対する $FeSO_4$ の促進作用は，$FeSO_4$ が子葉鞘へのオーキシンの侵入速度を高めるため，という考えがもたらされた。

ここで，屈曲試験に対する $FeSO_4$ の促進作用が，$FeSO_4$ が子葉鞘へのオーキシンの侵入を促進する，ということによる可能性の大きいことが示されたので，$FeSO_4$ をアベナ屈曲試験の感度をあげるために利用することは，未知の生長物質の検出に際しても有効な手段であるといえよう。

菌類の遺伝学的研究 VIII
トフンタケの交配系*

武　丸　恒　雄**

Tsuneo TAKEMARU**: Genetical Studies on Fungi VIII. Some Facts on the Mating System of *Psilocybe coprophila* Fr.

1959 年 1 月 6 日受付

[illegible]い くつかの報告がある。Gilmore[1] ならびに Brodie[2] によれば本菌の交配系は二極性であるが，一方 Vandendries[3],[4] によれば四極性であるという。最近，木村氏[5] [illegible]味ある見解を発表している。[illegible]の有無のみをよりどころにすれば二極性の交配様が得られるが，一歩進んでクランフの量的な差異を考慮に入れれば本菌は四極性を示す，というのである。筆者は目下ある一つの立場から各種の帽菌類の交配系を分析中であり，一部はすでに発表したが,[6][7][8] その一環としてトフンタケの交配[illegible]

材料と方法

本実験には，木村勘二氏[5] がすでに供試されたKおよびTの2系統を用いた。筆者の願いによって，これらの系統を心よく御分譲くださった同氏に深く感[illegible]ます

実験方法は，筆者[illegible]行なったのと全く同じであるから，重複をさけ[illegible]

実験結果と考察

i) 被覆四極性（クランプ形成能）: T系統の子実体から分離された 13 [illegible]，2 個体づつあらゆる組合わせで交配して，クランプ形成の有無ならびにその状態を調査した結果は，Table 1 a に示された通りである。“+” はクランプの形成が，交配された両菌糸体の接触部はもち[illegible]完全二核化を表わし，“(+)” はクランプの形成が接[illegible]まれない限定二核化を示している。“−” はいずれ[illegible]組合わせを表わしている。この Table から明らかな[illegible]合わせ（$A^3B^1 \times A^1B^1$ および $A^3B^1 \times A^1B^1$）では[illegible]テロ，B 因子がホモの illegitimate 組合わせ（$A^3B^1 \times A^1B^1$ および $A^3B^1 \times A^4B^1$）においても限定二核化が規則正しく営なまれるのである。それ故，クランフ形成の有無だけをよりどころにすれ[illegible]成の “状態”，いいかえれば完全二核化か限定二核化かの別をも考慮に入れれば四極性になるのである。さきに筆者がエノキタケ[6][7] およびウシグ[illegible]“被覆四極性” が，[illegible]トフンタケにおいても観察されたわけである。K 系統においても，Table 2 でみられるように，同様な被覆四極性の交配模様が得られた。

この事実を，前述の木村氏[5] の報告と考え合わせると[illegible]A 因子がヘテロ，B 因子がホモ[illegible]

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Table 1. a) Clamp formation from all possible matings between 13 monosporous mycelia from a fruit-body of T-stock. b) Fruit-body production from all clamp-positive pairings and from 13 monosporous mycelia themselves. Explanation of signs for this and following tables: "+" denotes complete dikaryotization on either side of the contact zone between two mated mycelia; "(+)", limited dikaryotization found only in the contact zone; "−", negative clamp-forming reaction; "B", appearance of barrages; "F", perfectly developed fruit-bodies bearing abundant basidiospores; "f", half-developed fruit-bodies with scanty spores; "r", fruit-body rudiments.

a

	1	5	6	10	12	7	8	9	3	4	11	2	13	
1	−	−	−	−	−	+	+	[illegible]	−	−	−B	(+)	(+)	
5	−	−	−	−	−	+	+	[illegible]	−B	−B	−B	$(+)^{B}$	$(+)^{B}$	
6	−	−	−	−	−	+	+	+	−B	−B	−B	$(+)^{B}$	$(+)^{B}$	A^3B^1
10	−	−	−	−	−	+	+	+	−B	−B	−	$(+)^{B}$	(+)	
12	−	−	−	−	−	+	+	+	−	−	−B	$(+)^{B}$	(+)	
7	+	+	+	+	+	−	−	−	$(+)^{B}$	$(+)^{B}$	(+)	−	−B	
8	+	+	+	+	+	−	−	−	(+)	$(+)^{B}$	(+)	−B	−	A^1B^1
9	+	+	+	+	+	−	−	−	(+)	$(+)^{B}$	$(+)^{B}$	−B	−B	
3	−	−B	−B	−B	−B	$(+)^{B}$	(+)	(+)	−	−	−	+	+	
4	−	−B	−B	−B	−	$(+)^{B}$	$(+)^{B}$	$(+)^{B}$	−	−	−	+	+	A^3B^1
11	−B	−B	−B	−	−B	(+)	(+)	$(+)^{B}$	−	−	−	+	+	
2	(+)	$(+)^{B}$	$(+)^{B}$	$(+)^{B}$	$(+)^{B}$	−	−B	−B	+	+	+	−	−	A^4B^1
13	(+)	$(+)^{B}$	$(+)^{B}$	(+)	(+)	−B	−	−B	+	+	+	−	−	

A^3B^1 A^1B^1 A^3B^1 A^4B^1

b

	1	5	6	10	12	7	8	9	3	4	11	2	13	
1	r					F	F	F				f	f	
5		f				F	F	F				f	F	
6			r			F	F	F				f	f	A^3B^1
10				f		F	F	F				f	F	
12					r	F	F	F				F	f	
7	F	F	F	F	F	f			F	f	f			
8	F	F	F	F	F		r		F	f	f			A^1B^1
9	F	F	F	F	F			f	F	f	f			
3						F	F	F	f			F	F	
4						f	f	f		r		F	F	A^3B^1
11						f	f	f			F	F	F	
2	f	f	f	f	F				F	F	F	f		A^4B^1
13	f	F	f	F	f				F	F	F		r	
	1	5	6	10	12	7	8	9	3	4	11	2	13	

の illegitimate 組合わせにおいても，クランプを規則的に形成することはすでに木村氏も観察しているが，さらに同氏は，この組合わせで形成されるクランプは正常の和合性組合わせの場合にくらべてその数が少ないことを見出し，これをよりどころにして両者を区別することによって四極性の交配模様を得たのである。このように木村氏は，形成されたクランプの"量的差異"を根拠としているのに対して，筆者はむしろクランプ形成（いいかえれば二核化）の"状態"をよりどころにし

Table 2. Mating pattern in K-stock of *Psilocybe coprophila*.

	1	2	4	5	9	3	13	7	10	11	6	8	12	
1	−	−	−	−	−	+	+	−B	−B	−B	(+)	(+)	(+)	A^1B^1
2	−	−	−	−	−	+	+	−B	−B	−	(+)B	(+)B	(+)	
4	−	−	−	−	−	+	+	−B	−B	−	(+)B	(+)	(+)	
5	−	−	−	−	−B	+	+	−B	−B	−B	(+)	(+)	(+)	
9	−	−	−	−B	−	+	+	−B	−B	−	(+)B	(+)	(+)B	
3	+	+	+	+	+	−	−	(+)B	(+)B	(+)	−B	−	−B	A^2B^2
13	+	+	+	+	+	−	−	(+)	(+)	(+)B	−	−B	−	
7	−B	−B	−B	−B	−B	(+)B	(+)	−	−	−	+	+B	+	A^1B^2
10	−B	−B	−B	−B	−B	(+)B	(+)	−	−	−	+	+	+	
11	−B	−	−	−B	−	(+)	(+)B	−	−	−	+	+	+B	
6	(+)	(+)B	(+)B	(+)	(+)B	−B	−	+	+	+	−	−	−	A^2B^1
8	(+)	(+)B	(+)	(+)	(+)	−	−B	+B	+	+	−	−	−	
12	(+)	(+)	(+)	(+)	(+)B	−B	−	+	+	+B	−	−	−	
	A^1B^1					A^2B^2		A^1B^2			A^2B^1			

て本菌の被覆四極性をみたわけである。交配された両菌糸体の接触部にのみクランプの形成が局限される限定二核化の場合は，どの部位にもクランプ形成がみられる完全二核化の場合にくらべて，[illegible]ある。このようにみてくると，筆者の観察は，すでに[illegible]氏が見出したと同じ現象を，異なる立場から[illegible]者の観察では，上記の illegitimate 組合わせの[illegible]接触部におけるクランプ数と量的に区別の困難な場合もあったことを附記しておく。

ii) Barrage 現象： ウシグソヒトヨの場合には，系統によって若干の差異はあるが，一般に barrageの出現が規則的で，限定二核化を伴なう組合わせでは必ずといっていい程この現象がみられることは既に報告した通りである[8]。ところがトフンタケにおいては，Table 1a（T 系統）および2（K系統）で示されているように，barrage（"B"）は必ずしも限定二核化に伴なわず，むしろ A 因子ホモ，B 因子ヘテロの illegitimate 組合わせの方に多く出現する傾向さえみられる。また A, B ともにヘテロの和合性組合わせでも，さらに同一交配型同士の間でさえも少数ながら barrage が観察された。なお Table 3a に示されている交配では，限定二核化の組合わせにおいて barrage が余りみられなく，むしろ接触部が幾分盛上[illegible]ていることが注目された。このように，トフンタケの場合には barrage の出現は，ウシグソヒトヨのように一定の規則に従うのではなく，[illegible]であり，また出現したbarrageの様子も[illegible]の場合とはいくらか異なり，一般に微弱である。

iii) [illegible]：T 系統について，完全，限定の別は問わず，とにかく二核化を行なった組[illegible]について子実体形成の有無とその状態を調査した結果が，Table 1b に示されている。なお本菌では単胞子起原の菌糸体が単独で子実体を[illegible]，この点に[illegible]子実体は一般に無定形で，キノコらしい整のった形を示さないが，成熟した正常の子実体は無数の担子胞子を着生し，菌傘部が黒色ないし濃紫色を呈する。このような子実体は Table 中に "F" で示されている。"f" は胞子数が僅少で菌傘部が白色の子実体を表わし，"r" は子実体の原基のみを形成してその後の発生がみられない場合を示している。Tableから明らかなように，完全二核化を伴なう和合性組合わせではすべて正常な子実体が発生しているのに対して，限定二核化を行なう A ヘテロ，B ホモの illegitimate組合わせでは正常な子実体を形成するものも幾組か存在するが，他の多くは胞子の形成がきわめて不良である。なお，*11* で示される

Table 3. a) Mating pattern between 12 monosporous mycelia derived from an illegitimate combination of T-stock, $3(A^3B^4)\times 8(A^4B^1)$. b) Clamp-forming reaction of these illegitimate F_1-mycelia with 4 testers of the parental stock.

a

	1	3	4	7	8	11	2	5	6	9	10	12	
1	−	−	−	−	−	−	(+)	(+)	(+)	(+)	(+)	(+)	A^3B^4
3	−	−	−	−	−	−	(+)	(+)	(+)	(+)	(+)	(+)	
4	−	−	−	−	−	−	(+)	(+)	(+)	(+)	(+)	(+)	
7	−	−	−	−	−	−	(+)	(+)	(+)	(+)	(+)	(+)	
8	−	−	−	−	−	−	(+)	(+)	(+)	(+)	(+)	(+)	
11	−	−	−	−	−	−	(+)	(+)	(+)	(+)	(+)	(+)	
2	(+)	(+)	(+)	(+)	(+)	(+)	−	−	−	−	−	−	A^4B^1
5	(+)	(+)	(+)	(+)	(+)	(+)	−	−	−	−	−	−	
6	(+)	(+)	(+)	(+)	(+)	(+)	−	−	−	−	−	−	
9	(+)	(+)	(+)	(+)	(+)	(+)	−	−	−	−	−	−	
10	(+)	(+)	(+)	(+)	(+)	+	−	−	−	−	−	−	
12	(+)	(+)	(+)	(+)	(+)	(+)	−	−	−	−	−	−	
	A^3B^4						A^4B^1						

b

Testers	1	3	4	7	8	11	2	5	6	9	10	12	
1	−	−	−	−	−	−	+	+	+	+	+	+	A^3B^4
7	(+)	(+)	(+)	(+)	(+)	(+)	−	−	−	−	−	−	A^4B^4
3	−	−	−	−	−	−	(+)	(+)	(+)	(+)	(+)	(+)	A^3B^1
2	+	+	+	+	+	+	−	−	−	−	−	−	A^4B^1

単胞子菌子体は，単独で胞子の豊富な正常子実体を形成した。

K系統においても，上記のT系統と全く同じ傾向がみられた。

iv) 子実体分析： 和合性組合わせから発生した子実体を分析すると，ふたたび Table 1a および2でみられるような被覆四極性を示す。また，上記の単胞子菌糸体 (*11*) が形成した子実体からはその菌糸体の交配型と同じ型をもつ一種類の胞子のみを形成する。さて，illegitimate の組合わせに生じた子実体にはどのような交配型をもつ胞子が形成されるだろうか。個々の胞子の交配型を分析する前に，形成された子実体が *11* でみられたような単相起原のものであるか否かを知るために，illegitimate 起原の F と f のすべてについて，まず多胞子培養を行なった。この培養にクランプが形成されなければ，単相起原と断定しても差支えないからである。その結果，f からの多胞子培養ではいづれもクランプが観察されなく，この子実体が単相であることを物語っているが，一方 F からの多胞子培養では明らかにクランプの形成がみられた。そこで次に，後者の子実体につくられた個々の胞子の交配型を分析したところ，総計42単胞子菌糸体についての観察では，いづれも両親のもつ交配型と同じ2種類の型のものばかりであった。その一つの例が Table 3 に示されている。これはT系統の illegitimate 組合わせ $3(A^3B^4)\times 8(A^4B^1)$ より生じた子実体の分析結果である。Table 3a は，分離された 12 単胞子菌糸体の総当り交配の模様を示している。この場合には限定二核化が規則正しく行なわれ，完全二核化はまったくみられない。また Table 3b は，これら 12 個体を4テスタ菌糸体（各交配型を代表）に戻交配した場合の交配模様である。これらの表から明らかなように，上記の illegitimate 起原の子実体からは，その両親と同じ交配型 (A^3B^4 と A^4B^1) をもつ2種類の胞子が発生したわけである。

一方，木村氏[11]の分析では，このような illegitimate 起原の子実体からは，両親のうちのどちらか一方の交配型をもつ1種類の胞子しか見出されていない。しかしこれは，illegitimate 起原の子実体がすべて単相であるというのではなくて，分析された子実体がたまたま f で示されるような単相起原のものであったというに過ぎないのである。

Summary

The mating system of *Psilocybe coprophila* was analyzed, using K-and T-stocks. As shown in Tables 1a and 2, in legitimate matings, clamps were observed microscopically both in the contact zone between two mycelia and on either side of it. Clamps were also found in all common *B*-factor matings, but in these pairings the formation of clamps was restricted to the contact zone only. Therefore, when only the contact zone is examined for clamps, a bipolar mating pattern is obtained. However, when the mycelia on either side of it are also tested for clamps, tetrapolarity is unmasked.

All matings where clamp-bearing hyphae had been observed were tested for their capacity to produce fruit-bodies under the same culture conditions. The same test was also done for individual monosporous mycelia. Some of the mycelia gave rise to haploid fruit-bodies bearing ample basidiospores, all of which were of the same mating type as the parent mycelia. As shown in Table 1b, perfectly developed fruit-bodies with abundant spores were obtained not only in all legitimate matings but also in some common *B*-factor matings. The fruit-bodies from legitimate matings produced spores of all four mating types; whereas, well-developed fruit-bodies from common *B*-factor matings produced only spores of the two parental types (Table 3b). Pairings between the monosporous mycelia derived from common *B*-factor matings showed bipolar pattern, where clamps were found only in the contact zone, but never on either side of it (Table 3a).

Barrages are irregularly manifested in common *A*-factor matings, in common *B*-factor matings, and in other matings (Tables 1a and 2). Thus, the barrages in this fungus seem to be of haphazard appearance but not of heritable characters.

文　　　献

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Penicillium islandicum Sopp.の培養中における菌体色素群の消長について

林　孝三*・菊池正彦*・岡本好正*

Kôzô HAYASHI, Masahiko KIKUCHI and Yoshimasa OKAMOTO: Studies on Pigment Formation in *Penicillium islandicum* Sopp. during Cultivation.

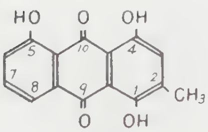
1959 年 2 月 3 日受付

Penicillium islandicum Sopp. の菌糸に形成される色素の種類およびそれらの化学構造については，すでに Howard and Raistrick[1] の研究があり，さらに Shibata *et al.*[2,3,4,5,6,7,8] は同種 NRRL 1175, 1036, N. I. 2699 の 3 株について詳細な研究を行われ，現在までに上記のようなアントラキノン系色素の存在が明らかにされた。

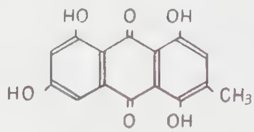
著者らは，これらアントラキノン系色素相互間の生成的関連を明らかにして，生合成機序の研究に資するため，*Penicillium islandicum* Sopp. を用いて，培養中に形成される各色素成分の消長について予備的研究を行ない，若干の知見を得たのでここに報告する。

材料および方法

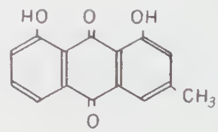
I　供試菌株：長尾研究所から分与された *P. islandicum*, NRRL 1175 株を用いた。



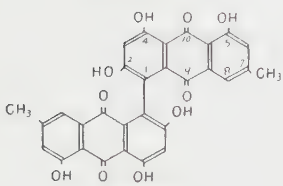
Islandicin



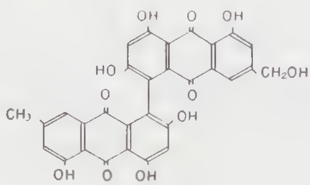
Catenarin



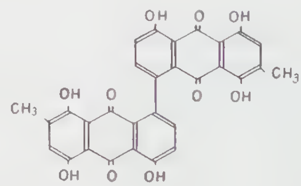
Chrysophanol



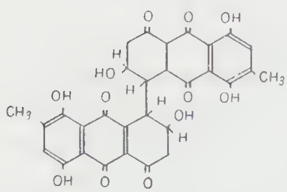
Skyrin



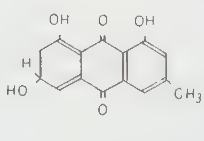
Oxyskyrin



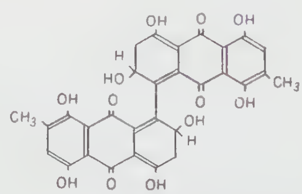
Iridoskyrin



Rubroskyrin



Flavoskyrin



Luteoskyrin

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Ⅱ　培養基

1)　完全培地

a)　固体培地：麦芽エキス (Bg. 1°)＋グルコース(3%)＋ペプトン(0.1%)＋寒天(2%)。

b)　液体培地：上記の固体培地から寒天を除いたもの。

2)　最少培地

a)　固体培地：Czapek-Dox 培養基(＋ショ糖(3%)＋寒天(2%))。

b)　液体培地：上記の固体培地から寒天を除いたもの。

Ⅲ　培養：定温器中 27°±2° で行なった。

Ⅳ　色素の同定：ペーパークロマトグラフィによる。新鮮な菌体をアセトンで抽出し，東洋ろ紙 No. 3 [illegible] ガラス円筒(4×40cm.) 中アセトン/ベンジン/水 (5:5:3.5) の上層を用いて上昇法一次元展開，風乾後酢酸マグネシウム(0.5%)のメタノール溶[illegible] R_f 値を測定した。各色素の同定には東大薬学部柴田教授から提供された結晶標品を対照として用いた。

実験および結果

以下の実験成績はいずれも 5～6 回の実験結果をまとめたものである。

Ⅰ　供試菌株の生産する色素

液体完全培養基(pH 5.8～6.0) 30～40 ml. ずつを分注した三角フラスコ(100 ml. 容)での液体培養，または，固体完全培養基(pH 5.8～6.0) 10～15 ml. ずつを分注した試験管(2×20 cm.)で 27° で 10 日間斜面培養した菌体をアセトンで抽出し，ペーパークロマトグラフィで菌体色素を同定した。(これらの培養ではつねに同一の結果が得られることが判ったので，以後の色素抽出には随時液体培養または斜面培養による菌体をもちいた)。著者らの結果を Shibata *et al.*[2] の結果と比較して表1に示す。

著者らの結果では Shibata *et al.*, の検出した Erythroskyrin 群中の R_f 0.63 を示すものと R_f 0.29 を示す未知色素との存在は認められなかった。したがって，著者らの用いた菌株は Chrysophanol, R_f 0.80 の色素 (以後 pigment-0.8 と呼ぶ), R_f 0.58 の Erythroskyrin, Flavoskyrin, Skyrin, Oxyskyrin, pigment-C の 7 種の色素を生産するものとして実験を進めた。

Ⅱ　培地の pH による色素形成の消長

菌体における色素形成が培地の pH によって影響されるか否かを検べるために，斜面完全培地 (pH 5.8～6.0) 上 27°, 10～15 日培養の分生胞子を，次のごとく，種々の pH 値を有する培地に 27° で斜面培養した。すなわち培地には完全，最

Table 1. Pigments detected in the mycelium of *P. islandicum*, NRRL 1175.

R_f-value	Pigments detected by Shibata *et al.* (1955).	Pigments detected by the present authors
0.97	Chrysophanol	+
0.80	unknown pigment	+
0.63	Erythroskyrin (structure unknown)	−
0.58	〃	+
0.49	Flavoskyrin	+
0.42	Skyrin	+
0.29	unknown pigment	−
0.16	Oxyskyrin	+
0.01	pigment-C	+

少の 2 者を用い，その pH 系列は

完全培地：pH 5.4, 5.8, 6.2, 6.6, 7.6

最少培地：pH 5.4, 5.8, 6.2, 6.6, 7.4

これら培地上での菌糸の生育および色素形成についての所見は次の通りであった。

1) 完全培地上での培養

i) Incubation 2 日――pH 5.4, 5.8, 6.2 の 3 者が他の 2 者より生育がやや良好である。色素の形成，分生胞子の形成はともにみられない。

ii) Incubation 4 日――色素形成は進行し，分生胞子も形成される。生育，色素形成ともに pH 5.4, 5.8, 6.2 の 3 者がやや良好であるがとくにいちじるしい差はみとめられない。

iii) Incubation 4～7 日――生育などの所見は ii) とほとんど同様である。このころには気菌糸の生育，色素の形成はおおむね頂点に達し，以後は気菌糸の老化が見えはじめる。

iv) Incubation 9 日――7 日目ごろとほとんど変りがない。

2) 最少培地上での培養

完全培地上のものに比較して生育がやや遅く，画線した部位に盛り上るようにして集落をつくる。また集落にいちじるしく水滴が出現し，色調は完全培地上のものにくらべてやや灰白色をおびる。

i) Incubation 2 日――pH 7.4 の場合は生育がやや劣るようである。

ii) Incubation 4 日――pH 5.4, 5.8, 6.2 の 3 者は色調が橙赤色であるが，他の 2 者は帯黄橙赤色である。水滴が大粒化してくる。

iii) Incubation 7 日――pH 7.4 の培養が他よりも生育はやや劣るが，色素形成においては 5 者とも区別しがたくなる。

iv) Incubation 9 日――水滴がつぶれて火口状の陥没を生じ，水滴は管底にたまる。集落の表面は硬化してくる。

10 日後各培地上の菌体を採取し，直ちにアセトンで抽出して色素成分をペーパークロマトグラフィでしらべた（表 2)。

また，この各 pH の各培地上に形成された分生胞子をそれぞれ同じ pH の同じ培地へ移植し (2代目) その色素の消長をしらべた。さらに同様にして 3 代目における消長をみたが，これらの場合，結果は第 1 代目とまったく同じであった。

Table 2. Effect of pH of the medium on pigment formation in the mycelia of *P. islandicum* Sopp. NRRL 1175.

pH	Culture media*	Pigments**						
		Chry	pig-0.8	Eryth	Flavo	Sky	Oxysky	pig-C
5.4	C. M.	+	+	+	+	+	+	+
	M. M.	+	−	+	−	+	+	+
5.8	C. M.	+	+	+	+	+	+	+
	M. M.	+	−	+	−	+	+	+
6.2	C. M.	+	+	+	+	+	+	+
	M. M.	+	−	+	−	+	+	+
6.6	C. M.	+	+	+	+	+	+	+
	M. M.	+	−	+	−	+	+	+
7.6	C. M.	+	+	+	+	+	+	+
7.4	M. M.	+	−	+	−	+	+	+

* C. M.: complete medium, M. M.: minimal medium.

** Chry: chrysophanol, Ery: erythroskyrin, Flavo: flavoskyrin, Sky: skyrin, Oxysky: oxyskyrin, pig-C: pigment-C.

以上の結果から色素形成は pH 5.4〜7.6 の範囲では不変であり，最少培地における pigment-0.8 と Flavoskyrin との消失は pH の影響ではな[illegible]

III 最少培地上[illegible]消長

Flavoskyrin および pigment-0.8 の出現が，培地の栄養状態によって左右されると思われるので，完全培地上で生じた分生胞子を最少培地へ移植し，10〜15日後に形成された分生胞子をさらに最少培地で10〜15日間培養し，それに形成された分生胞子を同様に最少培地へ移植する方法を反復[illegible]らべた(表 3)。

[illegible]ノの気菌糸が見られ，色素形成能の減退を示している。

IV 最少培地上の分生胞子を完全培地へ戻して培養した場合の色素形成の消長

実験IIIの結果から，菌の生育および色素形成が[illegible]培地上に形成された分生胞子も完全培地へ戻し培[illegible]るか否かをしらべたところ，表 4 の結果が得られた。

すなわち，菌を最少培地上で継代培養するとき，pigment-0.8 は 2 代目[illegible]，Flavoskyrin は 3 代目で形成されなくなるが，このころは完全培地へ

Table 3. Fate of mycelial pigments after successive inoculation on minimal medium.

Pigments	Culture*								
	C	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8
Chrysophanol	+	+	+	+	+	+	+	+	+
pigment-0.8	+	+	−	−	−	−	−	−	−
Erythroskyrin	+	+	+	+	+	+	+	+	−
Flavoskyrin	+	+	+	± or −	−	−	−	−	−
Skyrin	+	+	+	+	+	+	+	+	+
Oxyskyrin	+	+	+	+	+	+	+	+	+
pigment-C	+	+	+	+	+	+	+	+	+

* C: 10-day culture on complete medium.
M_1: 10-day culture on minimal medium obtained by inoculation with conidia of C.
M_2: Same as above obtained by inoculation with conidia of M_1.
M_3: Same as above obtained by inoculation with conidia of M_2.
………………………

表 3 から明らかのように，2 代目からは pigment-0.8 が消失し，3 代目から Flavoskyrin が消失する。また，8 代目になると Erythroskyrin が消失し，Chrysophanol, Oxyskyrin, Pigment-C の生成も判然としなくなる。なおクロマトグラム から推定される Skyrin の生産量も漸減し 8 代目ごろでは，その減少はきわめて顕著となる，このときの菌の外観は，気菌糸が少戻すと色素形成能を回復する。しかし，さらに最少培地上での培養を続けるとき，前者は5代目，後者は7代目で，その形成能は完全に失われ，完全培地へ戻してももはや回復しなくなる。

V 菌の発育過程における色素形成の順位

培養中における色素形成の順位については，すでに Shibata *et al.*[2] によって表 5 のように報告されている。

著者らは種々の培地を用いて，各色素の形成順

Table 4. Recovery of pigment formation in the mycelia, when the conidia formed on the minimal medium were transferred to the complete medium.

Pigments	Culture*													
	C	M_1	M_2	$M_2 \to M_3$	$M_2 \to C$	$M_3 \to M_4$	$M_3 \to C$	$M_4 \to M_5$	$M_4 \to C$	$M_5 \to M_6$	$M_5 \to C$	$M_6 \to M_7$	$M_6 \to C$	$M_7 \to M_8$	$M_7 \to C$
Chrysophanol	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+
pigment-0.8	+	+	−	−	+	−	+	−	+	−	−	−	± or	−	−
Erythroskyrin	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Flavoskyrin	+	+	+	−	+	−	+	−	+	−	+	−	+	−	−
Skyrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxyskyrin	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+
pigment-C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* C, M_1, M_2, M_3, ...M_7: c. f. foot note of Table 3.
$M_2 \to M_3$: Transfer of conidia of M_2-culture to the minimal medium.
$M_2 \to C$: Transfer of conidia of M_2-culture to the complete medium.
... .

Table 5. Sequence of pigment formation in the mycelia during cultivation on Czapek-Dox solution (after Shibata *et al.*, 1955).

Days after inoculation	Observation	Pigments formed*	pH
0			5.2
5	White colonies develop		
6	Reverse side of colonies: orange; Culture medium: pale yellow	Ery, Sky	3.1
7	Spore formation occurs; Reverse side of colonies: yellowish brown	Ery, Sky, Oxysky	3.1
8	Culture medium: reddish brown	Ery, Sky, Oxysky, Chry, pig-C	3.2
9	〃	Pigment formation is complete	3.2
10	〃		3.4
11	〃		3.6
12	〃		3.6
21	〃		4.0
25	〃		4.2

* Ery: erythroskyrin, Sky: skyrin, Oxysky: oxyskyrin, Chry: chrysophanol, pig-C: pigment-C.

位を詳細に検討した。培地は液体完全培地（pH 5.8 の培養液 40 ml. ずつを 100 ml. 容三角フラスコに分注），液体完全培地を 5 倍にうすめたもの（pH 6.2∼64. の培養液 40 ml. ずつを 100 ml. 容三角フラスコに分注），液体最少培地（100 ml. 容三角フラスコに pH 6.6∼6.8 の培養液 20 ml. ずつ分注のものと，40 ml. ずつ分注のもの）を用い，これらに斜面完全培地で得た分子胞子を接種して 27° に保ちつつ適当な時間ごとに菌体を採りペーパークロマトグラフィによって各色素の出現順位をしらべた。

1）完全培地上における色素の出現順位

栄養状態によって，pigment-0.8 および Flavoskyrin の形成はとくにいちじるしく影響され，最少培地上 1∼2 代の培養でそれらの形成が見られなくなる（表2，3）ので，まず完全培地を用い

Table 6. Sequence of pigment formation in the mycelia during cultivation on complete medium (40 ml. solution in each 100 ml. flask, pH 5.8—6.0, 27°.).

Days after inoculation	Number of Experiments	Observation	Pigments	pH
0				5.8—6.0
2	2	Mycelia cover the whole surface of the medium	Pigment formation is complete (pig 0.8 and Flavoskyrin are present in slightest amount)	4.4—4.6
3	2	Mycelia: orange to orange-red. Spore formation occurs	Pig-0.8 and Flavoskyrin clearly appear	3.0—3.2 3.4—3.6
4	2			2.6—2.8 2.8—3.0
5	1			2.4—2.6

Table 7. Sequence of pigment formation in the mycelia during cultivation on complete medium of 1/5-dilution (40 ml. solution in each 100 ml. flask, pH 6.2—6.4, 27°.).

Days after inoculation	Number of Experiments	Observation	Pigments formed*	pH
0				6.2—6.4
2	2	Colonies develop		4.4—4.6
3	2			3.8—4.0
4	2	Mycelia: yellow to yellowish orange; Spore formation occurs	Ery, Sky, Oxysky, Chry, pig-C	3.8—4.0
5	2	Mycelia: Orange to orange-red	Pigment formation is complete	3.4—3.6
6	1			3.2—3.4
8	1			3.4
10	1			3.4—3.6

* Ery: erythroskyrin, Sky: skyrin, Oxysky: oxyskyrin, Chry: chrysophanol, pig-C: pigment-C.

て実験した。結果は表 6 のとおりである。

この場合，接種後 3 日にして，すでに全色素の形成が完了するので，形成順位の決定は困難であった。しかし，pigment-0.8, Flavoskyrin の 2 者はクロマトグラム上の色がきわめて淡く，4 日後になってはじめてはっきりしてくるので，最も遅れて出現するものと思われる。

2) 5倍稀釈の完全培地上での色素の出現順位

完全培地そのものでは，全色素の出現完了が急速に過ぎて，形成順位の査定には不適当であったので，完全培地を 5 倍に稀釈して培養実験を行なった（表 7)。

この場合も全色素形成の完了は早いが，接種後 4日でまずErythroskyrin, Skyrin, Oxyskyrin, Chrysophanol, pigment-C が現われ，次いで 5 日目に pigment-0.8, Flavoskyrin が現われた。すなわち，後 2 者の形成がもっとも遅れることが確かめられた。

3) 最少培地上における色素形成の順位。

最少培地上では 1~2 代で pigment-0.8 および Flavoskyrin の形成が見られなくなる（表 2, 4) ので，この場合に用いる分生胞子の採取にはとくに注意を払って，液体完全培地上 5~7 日で菌の生長が頂点に達したころの分生胞子を用いた。

a) 100 ml. 容三角フラスコに 20 ml. ずつ分注した培地で培養した場合（表 8a)。

この場合，まず Erythroskyrin と Skyrin が現われ，ついで Oxyskyrin, 次に Chrysophanol と pigment-C，最後に pigment-0.8 と Flavoskyrin が出現することがわかった。

Table 8-a. Sequential occurrence of pigment components in the mycelia during cultivation on minimal medium (Czapek-Dox solution; 20 ml. solution in each 100 ml.-flask, pH 6.6—6.8, 27°).

Days after inoculation	Number of Experiments	Observation	Pigments formed*	pH
0				6.6—6.8
2	2	Colonies develop; Medium: colorless		5.0 4.4—4.8
3	2	Colonies cover the whole surface of the medium		3.6—3.8
4	4	Mycelia: slightly orange Medium: pale yellow	Ery, Sky	3.2—3.4 3.4—3.6
5	3	Mycelia: orange……… Mycelia: orange-red…	Ery, Sky, Oxysky Ery, Sky, Oxysky, Chry, pig-C	3.6—3.8
6	2	Mycelia: brownish orange-red	Ery, Sky, Oxysky, Chry, pig-C	3.2—3.4 3.6—3.8
7	2	Mycelia: brownish orange-red; Spore formation occurs	〃	5.4—5.6 5.8—6.0
10	2	Medium: brownish-yellow	Pigment formation is complete	6.6—6.8

* Ery: erythroskyrin, Sky: skyrin, Oxysky: oxyskyrin, Chry: chrysophanol, pig-C: pigment-C.

b) 100 ml. 容三角フラスコに 40 ml. ずつ分注した培地で培養した場合（表 8b)。

Table 8-b. Sequential occurrence of pigments in the mycelia during cultivation on minimal medium (Czapek-Dox solution: 40 ml. solution in each 100 ml.-flask, pH 6.6—6.8, 27°).

Days after inoculation	Number of Experiments	Observation	Pigments formed*	pH
0	2			6.6—6.8
2	2	Colonies develop		4.2—4.4
3	2	Colonies cover the whole surface of the medium		3.8—4.0
4	2	Mycelia: orange to orange-red	Ery, Sky, Oxysky	3.6—3.8
5	2	Color deepens	Ery, Sky, Oxysky, Chry, pig-0.8, pig-C	3.4—3.6
6	2	Medium: pale yellow	Pigment formation is complete	3.4—3.6
7	1	Spore formation	〃	3.4—3.6
9	1			4.2—4.4

* Ery: erythroskyrin, Sky: skyrin, Oxysky: oxyskyrin, Chry: chrysophanol, pig-C: pigment-C, pig-0.8: pigment-0.8.

この実験では，Erythroskyrin, Skyrin, Oxyskyrin が先に現われ，ついで Chrysophanol, pigment-C, pigment-0.8 が現われ，最後に Flavoskyrin が出現した。

表 8a, 8b の結果から，最少培地での培養において各色素の形成される順位は，最初に Erythroskyrin と Skyrin，ついで Oxyskyrin，次に Chrysophanol と pigment-C，次に pigment-0.8，最後に Flavoskyrin と結論される。Erythroskyrin と Skyrin との形成順位は決定することができなかった。[illegible]く黄～橙色に色づいたときには，すでに両者が共存しているからである。同様に Chrysophanol と pigment-C の形成もほとんど同時に行なわれる。

なお，表 6～8 で注目すべきことは，培地の pH 値の変動である。菌の生育につれて培地は酸性に傾き，色素形成のはじまるときには pH 3.0 前後となり，色素形成の極大または完了したと思われるところから pH 値は次第に上昇し，時にはまったく当初の pH 値まで[illegible]ることがある（表 8a）。

総括および考察

1）供試菌株に見られる色素の種類について Shibata *et al.*[2] は Erythroskyrin に 2 個のスポットすなわち，R_f 6.3, R_f 5.8 をあてているが，著者らの実験では R_f 6.3 のものは見られなかった。また R_f 0.29 の赤色色素も検出できなかった。これらの点については今後再検討を加えたい。

2）菌の色素形成は培地の pH（試験範囲 5.4～7.6）に影響されない（表 2）。このことは菌の生長につれて培地の pH が一旦低下し，ついでまた上昇すること（表 5, 6, 7, 8）からも推察される。菌がある培地上で生育するとき，ある種の物質（これがふたたび色素形成に利用されるか，あるいは菌糸体形成にともなう二次的生産物であるかについては不明）を生産して培地の pH を自律的に調整することが可能なために，当初の pH 値はあまり問題でないのかも知れない。

培地の pH 値の変動は色素形成および菌の生長と密接に関連し，pH 値の最小点は色素形成の極大または完了と一致する（表 5～8）。また，菌の生長においてもこの pH 値を境として，以後は菌糸の老化がはじまる（実験 II 参照）。

3）培地の栄養状態は色素形成を左右し，とくに pigment-0.8, Flavoskyrin はいちじるしく影響される（表 2～5）。したがって色素の生産を主眼

とする場合には，培地にじゅうぶん注意する必要がある。Czapek-Doxなどの培地を用いる際には，じゅうぶんな栄養条件下で得たもっとも新鮮な分生胞子を接種することが必要である。

4) 培養中，菌糸に形成される色素の出現順位(表 7〜9)については Shibata *et al.*[2] の結果 (表 6) とほとんど一致し，Erythroskyrinと Skyrin が最初に形成され，ついで Oxyskyrin，次に Chrysophanol と pigment-C，次に pigment-0.8，Flavoskyrin の順序である。

もっとも遅れて出現するpigment-0.8とFlavoskyrin とは栄養状態によっても簡単にその出現が左右されることから考えると，これらの合成は生合成の末端部に位置するものと思われる。

一方，Skyrin は Erythroskyrin とともに最も早期に出現し，最小培地上でも最後まで消失しないことから，これらはアントラキノン合成の主要な前階段にあり，かつ，もっとも安定な色素と考えられる。

Oxyskyrin が Skyrin についで出現することは，Skyrin の 7-CH_3 の酸化によって Oxyskyrin が生成することを暗示するものとして興味がある。Chrysophanol，pigment-C，pigment-0.8，Flavoskyrinについてはまだ何もいえない。

Erythroskyrin はまだその構造が明らかにされていないが，Erythroskyrin が形成されなくなってもSkyrin，Oxyskyrin などの形成が見られる (表 4) ことからすると，Skyrin 合成は Erythroskyrin を経由するものではないと考えられる。

本研究に用いた色素の結晶標品は東京大学薬学部 柴田承二 教授より，また菌株は長尾研究所の 椿 啓介氏より分与され，同時に有益な助言を賜わった。ここに厚く感謝の意を表する。

Summary

The formation of anthraquinone pigments in the mycelia of *Penicillium islandicum* Sopp. NRRL 1175 during cultivation on different kinds of media was studied in detail by means of paper chromatography.

1) Seven pigments, erythroskyrin, skyrin, oxyskyrin, chrysophanol, pigment-0.8, pigment-C and flavoskyrin, were detected in the mycelium grown on complete medium (Table 1).

2) Within a range of pH 5.4 to 7.6, the formation of the mycelial pigments did not depend on pH-value of the culture media used (Table 2). However, the occurrence of the pigments, in particular pigment-0.8 and flavoskyrin, was found to be affected by the nutrients added to the medium (Table 2, 3 and 4).

3) After several generations obtained by successive inoculation of conidia on the minimal medium, the biosynthetic capacity of the mold to form both pigment-0.8 and flavoskyrin is completely abolished, and can not be recovered even after inoculation on the complete medium (Table 4).

4) From the data obtained in relation to the sequence of pigment synthesis in the mycelia (Table 5-8), it is suggested that skyrin is a primary product in the biosynthesis of anthraquinone pigments and oxyskyrin is derived therefrom through oxidation of its 7-CH_3 group. Both pigment-0.8 and flavoskyrin are probably formed in the final step of biosynthesis.

5) Since the structure of erythroskyrin remains unsettled, any final evidence could not be obtained at present for the biosynthetic interrelationship between erythroskyrin and other mycelial pigments. However, in view of the fact that the

formation of skyrin takes place in growing mycelia even after erythroskyrin has ceased to be formed (Table 3 and 4), it is suggested that erythroskyrin is not involved in biosynthetic route of skyrin as an intermediate.

文　　献

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〔追　記〕この報文を本誌に投稿した後で，柴田教授の示唆により，pigment-0.8 と Emodin とをペーハーで[illegible]，R_f [illegible]ざる紙上での呈色試験において両者は完全に一致することが認め[illegible]，本[illegible] pigment-0.8 を Emodin [illegible]と[illegible]。詳細[illegible]追って報告する。

抄　　録

1. 開放植物細胞における原形質分離実験

Wartenberg, A., Plasmolyseversuche an geöffneten Pflanzenzellen. Ber. Deutsch. Bot. Ges. 70 (7): 263-274 (1957).

2. 開放植物細胞の原形質分離の問題について

Drawert, H., Zur Frage der Plasmolyse geöffneter Pflanzenzellen. ibid. 70 (8): 401-408 (1957).

3. 開放植物細胞の原形質分離

Wartenberg, A., Die Plasmolyse geöffneter Pflanzenzellen. ibid. 71 (4): 169-176 (1958).

原形質分離[illegible]についての紹介

1. ネギ類，シオグサ，フシナシミドロ，ホシミドロ，ア[illegible]など[illegible]され一端が開放されて膨圧のない細胞でも 100 の中 70 ぐらいまではほぼ完全に原形質分離を起こし，その[illegible]原形質分離[illegible]傷口に[illegible]こともあったがほとんどすべて傷口とは反対側の端にも見られた。原形質分離一般に適用する従来の古典的理論によれば，脱水により原形質体が収縮し膜粘着力が凝集力より小さくなってそのもっとも弱いところで細胞膜からひきはがされる…すなわち正の分離部位と部分的粘着によりヘヒトの糸がひかれる…とされていた概念とこのような現象とは調和できない。この場合膜粘着力の最小点は切断開放面であるはずで原形質体はこゝから細胞内へ縮みこまねばならないはずである。またはたして膜からひきはがされるほどの強い負圧が細胞内に生ずるかどうかにも疑問がもたれる。これはむしろ液胞の脱水収縮と同時に原形質体の外質が膨潤化し小胞化して内質と細胞膜との間を押しあけその小胞は次第に発達癒合し—これがいわゆる原形質分離腔である—その間を横切る原形質がいわゆるヘヒトの糸として見られるという新しい考えによって説明されるべきであると原形質分離の理論に関して一つの新説を提唱した。

2. これに対してタマネギの実験で傷害により傷口の原形質は[illegible]ではいちじるしく粘性が高まり膜粘着力も強められ膜からの剝離に対する抵抗は無傷の他端よりもずっと大きくなって，いわば原形質の栓となって傷面につまり負の分離部位となるのは当然であり，また螢光色素を加えての観察から原形質分離腔は負圧による剝離にもとずくことも明らかであって，従来の原形質分離の概念とまったく一致するもので何ら新規の仮説は必要でないことを強く反論した。

3. これに答えて，タマネギは実験の時期が問題であって生理的活性の高いそして傷害の影響の最小の時期に行なわれねばならず，冬眠状態に入りかけた材料では比較できない。生長期には傷面原形質の凝固や粘着力の増大による開口部の栓などはほとんど問題にならない。また分離液の濃度

の高い時には分離腔増大により切断開放口から原形質体が押しだされたり，正常細胞でも膜孔から原形質が外へつきだされる[illegible]され，この原形質分離腔はかなりの正圧をもつことは確かであって，これらの事実からそのような反[illegible]当であることを主張した。

（吉田吉男）

本会記事

名簿訂正

さきに作成いたしました会員名簿のなかで外国関係の名誉，外国通信会員の記載洩れがありましたので訂正いたします。これらの方は植物学会75周年記念大会（第22回大会）において名誉，外国通信会員に推薦された方々です

（名誉会員）

J. Bonner, E. Bünning, R. W. Chaney, H. St. John, 羅　宗洛, W. Ruhland, K. V. Thimann,

（外国通信会員）

E. R. Fosberg, B. Lindquist, 郑　万鈞, H. Ullrich

支部だより

（北海道支部）

2月例会（2月11日，北大・[illegible]）[illegible]：細胞伸長におよぼす[illegible]の作用機作，館脇操：北海道中央高地の[illegible]について

宮部先生記念講演会（4月25日，北大・農）田中一郎：欧米ところどころ，山田幸男：ヒトエグサ群について

なお北海道支部長には館脇操氏，[illegible]佐美正一郎，松浦一の両氏が選ばれました。

（関東支部）

5月例会（5月16日，東大・理）長谷川正男：シキミ酸のトランスフォーメーションについて。土屋毅：酵母の血清学的分類。

[illegible]お茶の水女子[illegible]

北陸支部

支部第三回大会（石川県加賀市・大聖寺高校，5月17日）吉池博：高等植物の進化の基本法則[illegible]小林卑美：シュロソウ属植物の生活史の研究，（1）根茎の形成と開花について。柴田万年，石倉成行：チューリップ花のアントチアンに[illegible]

（近畿支部）

三十三年度支部大会（5月17日，大阪大・理）田中長三郎：史学を背景とした柑橘の分類地理。広瀬弘幸，熊野茂：2，3の黄色鞭毛藻類に見られ[illegible]量に対するγ線の影響．滝本敦：朝顔の日長反応[illegible]

（九州支部）

第54回例会（4月18日，九州大・教養）茅野博：構造雑種におけるキアズマの研究。I. ノヒ[illegible]：[illegible]における放出四分胞子の構造。

なお九州支部の評議員には，細川隆英，野口彰，瀬川宗吉の3氏が選出されました。

Cytological and Morphological Studies on the Gametophytes of Ferns XII. Suction Force of the Wall Cell of Antheridium and of the Spermatid

by Isami IGURA*

[illegible]: シダ類の配偶体に関する細胞学的ならびに形態学的研究 XII.
造精器壁細胞および精細胞の吸水力

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Introduction

As regards the mechanism of the dehiscence of antheridium, many investigations were hitherto reported[1]. They were made principally from the morphological viewpoint, though some of them from more or less taxonomic standpoint. Fujii and Asahina[2] studied the discharges of the *Isoetes*-spermatozoids caused by chemicals. The dehiscence of antheridium of fern has, however, not yet been analysed cytomorphologically. Hiraoka[3] studied the suction force of the spore mother cell of *Cyrtomium* in meiosis and reported that an increase in suction force at the onset of the bouquet stage and a decrease in the pachytene stage. This decreased suction force is maintained till later stages. Recently Holden[4] studied the factor of dehiscence of flax-fruit, and found that the diffusion pressure deficit (DPD) shows a slightly greater value for the dehiscent variety than for the semidehiscent variety and the degree of imbibition has an effect on the amount of dehiscence. In order to understand the mechanism of the dehiscence of antheridium, it is important to measure the osmotic values of wall cells of the adult antheridium and of the spermatid, which in turn will give information about the magnitudes of their suction forces.

Materials and Methods

As experimental materials, the antheridia produced on the prothallia of the following ferns belonging to Eufilicineae were employed: *Leptogramma totta* J. Smith, *Matteuccia struthiopteris* Todaro, and *Osmunda cinnamomea* L. var. *asiatica* Fernald. Of these, however, the first one was chiefly used.

As for the determination of suction force, the principles and the methods of "Gleichgewichtsmethode" of Ursprung and Blum[5], Ursprung[6], and Hiraoka[3] were followed. The prothallia, on which the antheridia were produced, were dipped in a drop of liquid paraffin, and the minor diameters of the wall cells, i. e. the cap cell,

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the ring cell, and the basal ring cell, were measured by means of a micrometer (L). Then, the liquid paraffin was replaced by a series of aqueous solutions of sucrose whose concentrations were made from 0.50M to 0.80M with intervals from 0.01M to 0.02M. And as in the case of the liquid paraffin, the minor diameters of the wall cells were measured (L′). The immature antheridia, where the spermatoteleoses were going on, did not extrude the spermatids in the solution. Therefore, the mature antheridia which had finished the spermatoteleoses were selected for observation. As the form of wall cell is not suitable for the measurement of its volume, the diameter of the wall cell was regarded as the criterion showing the osmotic pressure of the wall cell.

When L was equal to L′ at a certain concentration (C_1) of the aqueous solution of sucrose, viz. the wall cell did not alter its form (volume) and no extrusion of the spermatids out of the antheridium occurred, the osmotic value C_1 was regarded as the suction force of the wall cell. The temperature of the solution was kept at $20\pm2°$. For the purpose of investigating the suction force, the experiment was conducted merely by using the solution of sucrose, and glucose, urea, KCl, $CaCl_2$ and $AlCl_3$ were used in order to obtain complementary data concerning this problem. The concentrations of these aqueous solutions were made up from 0.50M to 1.00M with intervals from 0.01M to 0.02M.

Now that the forms of the spermatids are almost spheric, the changes of their volumes are available to be measured by a method which is identical with that in the case of the wall cell. The volume of the spermatid within the central cavity of antheridium which was dipped in a drop of liquid paraffin on the slide glass, was measured by means of a micrometer (V). Next, the liquid paraffin was replaced by a series of aqueous solutions of sucrose of graded concentrations as media, and the volume of the spermatid extruded out of the antheridium or in the central cavity was measured (V′). When V and V′ are the same at a certain concentration (C_2), the osmotic value represented by this concentration of the sucrose solution was regarded as the suction force of the spermatid. According to the same measurement, the volumes of spermatids contained in the central cavity of the antheridium were also represented as v and v′, respectively.

Results

In the aqueous solution of sucrose 32 antheridia were examined in every solution and the wall cells of antheridia showed increases in their volumes. That is to say, the wall cells of antheridia swelled in some hypotonic solution (below 0.96M) (Fig. 1), though the swelling grades were not the same. As to the swelling grade, that of the basal ring cell is the greatest in many cases, and it may be assumed, in general, that the swelling grade of the basal ring cell is the greatest, that of the cap cell the smallest, and that of the ring cell intermediate (Table 1). This phenomenon is also observed in the experiments concerning glucose and urea. The results in the

case of *Leptogramma totta* were shown in Table 1. However, other species used in the present experiment showed also fundamentally the same results. In the case of sucrose, 0.74M aqueous solution makes the volumes of the wall cells increase and these cells swell. Then, the extrusion of the spermatids occurs. On the other hand, in the case of 0.98M solution, the wall cells swell and the spermatids extrude rarely out of the antheridium, but in many cases the wall cells do not change their volumes, namely they do not show their turgor pressures, consequently the extrusion of the spermatid does not arise. The wall cells show the convex plasmolyses and, of course, the spermatids do not extrude out of the antheridium in the case of 1.00M solution. Some representative results are given in Table 1.

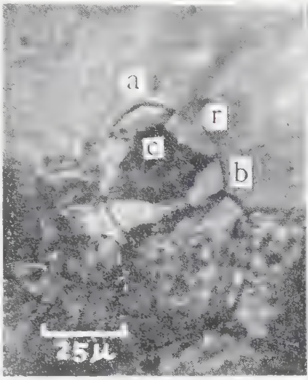

Fig. 1

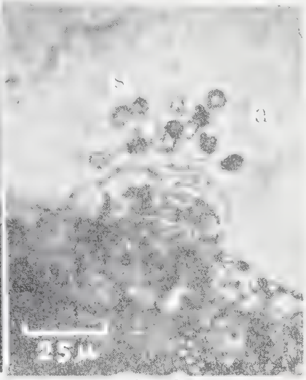

Fig. 2

Fig. 1. The wall cells of antheridium of *Matteuccia struthiopteris* Todaro which are increasing their volumes in 0.96M aqueous solution of sucrose (Phase contrast microscope, negative high).
a, cap cell; r, ring cell; b, basal ring cell; c, central cavity in which the spermatids are included.

Fig. 2. The spermatids extruded out of the central cavity of antheridium in *Osmunda cinnamomea* L. var. *asiatica* Fernald in redistilled water (Phase contrast microscope, negative high).
a, detached cap cell; s, spermatids out of which the spermatozoids will be extruded in a moment.

From these results it is presumed that the suction force of the wall cell corresponds to the osmotic value of 0.98M aqueous solution of sucrose, which is calculated to be 33.6 atmospheres in *Leptogramma totta*. According to the same presumption, the suction force is supposed to correspond to the osmotic value of 0.85M and 0.92M aqueous solution of glucose and urea, respectively. These osmotic values represented as suction forces of the wall cells are generally greater than those of the prothallial cells which are vegetative. For instance, in the case of sucrose, the greatest osmotic value of the prothallial cell in VI region (apical region) was 0.82M aqueous solution[7]. This fact is considered to be worthy of notice.

Concerning KCl, $CaCl_2$, and $AlCl_3$, some representative results are shown in Table 2.

From Table 2, the suction force of the wall cell is supposed to correspond to the osmotic value of 0.76M aqueous solution of KCl in *Leptogramma totta*. It is similarly found that, in $CaCl_2$ and $AlCl_3$ solutions, the osmotic values of 0.61M and 0.58M aqueous solutions were regarded as the suction force of the wall cell, respectively. These values are greater than the osmotic values of the prothallial cells as in the case of

Table 1. Increase of the diameter of the wall cells of the antheridium in *Leptogramma totta* J. Smith. (1).

Solution	Concentration (M)	Increase of the diameter of the wall cell (L′−L)/L×100			Extrusion of spermatids out of the antheridium
		Cap cell	Ring cell	Basal ring cell	
Sucrose	0.96	150.0	166.6	166.6	Extrusion
		166.6	200.0	208.3	〃
		207.7	258.3	233.3	〃
		215.3	275.0	280.0	〃
	0.98	130.0	146.9	150.0	〃
		0.0	0.0	0.0	No extrusion
	1.00	0.0	0.0	0.0	〃
		Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	〃
Glucose	0.83	113.3	169.2	169.2	Extrusion
		207.0	258.3	269.2	
	0.85	84.6	100.0	100.0	No extrusion
		0.0	0.0	0.0	〃
	0.87	0.0	0.0	0.0	〃
		Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	〃
Urea	0.90	130.7	169.2	176.9	Extrusion
		215.3	246.1	275.0	〃
	0.92	50.0	53.3	53.3	No extrusion
		0.0	0.0	0.0	
	0.94	Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	〃

sucrose. For example, in $CaCl_2$ solution the greatest value of the prothallial cell in VI region (apical region) of *Leptogramma totta* was 0.44M aqueous solution.

The representative results with respect to the spermatids shown in Table 3 were obtained.

As the materials, 132 spermatids produced in 37 antheridia were employed and the spermatids were observed to be osmotically in equilibrium with 0.74M aqueous solution of sucrose used as a medium (Table 3). That is, this concentration of the sucrose solution shows the osmotic value which corresponds to the suction force and equals to 23.1 atmospheres in *Leptogramma totta*. This value is considerably high. A possible consideration is able to be advanced below from the results of Table 3. When the antheridium was immersed in the water, the increases of the volumes of the spermatids contained in the central cavity of the antheridium occur more or less owing to their suction forces. After the extrusion of the spermatids out of the antheridium, the spermatids gradually increase their volume (Fig. 2) and reach the maximum of increasing, and then the spermatozoids come out of the spermatids. This high suction force is considered to be an important factor which plays a funda-

Table 2. Increase of the diameter of the wall cell of antheridium in *Leptogramma totta* J. Smith. (2).

Salt	Concentration (M)	Increase of the diameter of the wall cell $(L'-L)/L\times100$			Extrusion of spermatids out of the antheridium
		Cap cell	Ring cell	Basal ring cell	
KCl	0.74	100.0	130.7	130.7	Extrusion
		166.6	207.2	208.0	
		208.8	284.6	284.6	
	0.76	33.3	44.6	33.3	No extrusion
		0.0	0.0	0.0	〃
	0,78	Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	
$CaCl_2$	0.60	100.0	113.3	114.2	Extrusion
		207.7	233.3	246.1	
	0.61	17.8	19.2	20.0	No extrusion
		0.0	0.0	0.0	〃
	0.62	0.0	0.0	0.0	
		Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	
$AlCl_3$	0.56	115.3	130.7	140.0	Extrusion
		203.3	233.3	246.1	
	0.58	100.0	115.3	115.3	No extrusion
		0.0	0.0	0.0	
	0.61	0.0	0.0	0.0	
		Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	

Table 3. Increase of the volume of spermatid in *Leptogramma totta* J. Smith.

Solution	Concentration (M)	Spermatid in the central cavity of antheridium $(v'-v)/v\times100$	Spermatid extruded out of the antheridium $(V'-V)/V\times100$	Extrusion of the spermatozoid
Sucrose	0.72	0.0	30.9	Extrusion
		12.5	45.2	〃
		14.3	58.2	
		19 0	61.7	
		21.2	72.8	
	0.74	0.0	27.1	〃
		0.0	0.0	No extrusion
	0.76	0.0	0.0	〃
		Shrinkage of the spermatid-membrane occurs slightly	Shrinkage of the spermatid-membrane occurs slightly	〃
Redistilled water		0.0	40.5	Extrusion
		13.2	58.2	〃
		24.9	103.4	〃

mental role in the dehiscence of the antheridium and in the extrusion of the spermatozoid out of the spermatid.

Discussion

It has become evident that the wall cells of the antheridia in *Leptogramma totta* and other two species exhibit considerable high suction forces and are able to increase their volumes. *Leptogramma totta* shows a suction force corresponding to the osmotic value of 0.98M aqueous solution of sucrose which is equivalent to 33.6 atmospheres. Besides sucrose, the suction forces which corresponded to 0.74M, 0.61M, 0.58M, 0.92M, and 0.85M aqueous solutions of KCl, $CaCl_2$, $AlCl_3$, urea, and glucose were obtained, respectively. It can also be seen that the osmotic value of the wall cell is higher than that of the prothallial cell. It is known that the osmotic value of the seashore plant is high[8], while that of the alpine plant is, in general, low[9]. In the present study the osmotic value of the wall cell of the antheridium and the spermatid have been confirmed to be higher than or almost equal to that of the seashore plant. Diannelidis[10] reported that the osmotic value of the guard cell of the stoma is higher than that of the epidermal cell in *Stratiotes*. The same fact has been also observed between the antheridium and the prothallial cell. The present writer considers that, as the guard cell which has the function of opening and closing has a high osmotic value, so do the wall cells of the antheridium also possess a high activity of swelling owing to their big osmotic value.

The spermatids contained in the central cavity of the antheridium increase more or less their volumes in many cases and those extruded out of the antheridium increase markedly in the water. And the suction force was supposed to correspond to the osmotic value of 0.74M aqueous solution of sucrose (23.1 atmospheres) in *Leptogramma totta*. This value is comparatively large as described above and it is considered that this magnitude of the suction force of the spermatid may be related to its extrusion out of the central cavity of the antheridium.

Therefore, confirmation of the suction force of the wall cell and that of the spermatid is significant in clarifing a factor in the dehiscence of the antheridium, i. e. the extrusion of the spermatid out of the antheridium, and the discharge of the spermatozoid out of the spermatid.

Summary

Surveying the suction forces of the wall cells, viz. the cap cell, the ring cell, and the basal ring cell of the antheridium and the suction force of the spermatid chiefly in *Leptogramma totta* J. Smith, the following facts were obtained.

1. The suction force of the wall cell is comparatively high and is equivalent to the osmotic value of 0.98M aqueous solution of sucrose which corresponds to 33.6 atmospheres.

2. This value is higher than that of the seashore plant cell known as possessing

a high osmotic value. Concerning the magnitude of the osmotic value, the relationship between the wall cells and the prothallial cells resembles the relationship between the guard cells of stomata and the epidermal cells.

3. The suction force of the spermatid is also comparatively high and corresponds to the osmotic value of 0.74M aqueous solution of sucrose (23.1 atmospheres).

4. The mechanism shown by the wall cell and the spermatid having a high suction force is considered to be an important factor in the dehiscence of the antheridium and the extrusion of the spermatozoid out of the spermatid.

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摘　　　要

ミゾシダ (*Leptogramma totta* J. Smith) を主な材料とした外，2 種のシダを材料として，それらの前葉[illegible]吸水力を Ursprung and Blum などの "平衡法" を適用してしらべた結果は次のように要約できる。

1. [illegible]大きく，ミゾシダでは蔗糖の 0.98 モル[illegible]浸透価，すなわち 33.6 気圧に[illegible]する。

2. この値は高い浸透価を有する海岸植物の浸透価より高い。そして造精器の壁細胞の浸透価は前葉体細胞[illegible]それよりも高いという事実に[illegible]

3. 精細胞の吸水力も比較的大きく，ミゾシダでは蔗糖の 0.74 モル水溶液 (23.1 気圧) に相当する吸水力がある。[illegible]

4. [illegible]ことは，それ[illegible] activity [illegible]高いことを示すものであり，造精器裂開や，精細胞からの精子の脱出の主要な一因子であると考える。

Fruit Body Formation of Red Bread Mold *Neurospora crassa* III. Effect of Nitrogen Sources with Special Reference to the Ionic Ratio of Ammonium and Nitrate in the Medium

by Taro ITO*

伊藤太郎： アカパンカビの子実体形成 III. 窒素源の影響，とくに培地中における アンモニウム塩と硝酸塩のイオン比について

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In order to analyze the physiological mechanism of sexual reproduction of heterothallic fungi, *Neurospora crassa,* as an endogenous factor, the hormonal effects of the culture filtrates of both mating type strains on the formation of fruit body should be studied.

In the vegetative stage the mycelial and conidial development of the vegetative thallus is achieved by hyphal tip elongation and somatic hyphal cell division. In the reproductive stage, the fruit body formation is initiated by gamentangial copulation of vegetative hyphae accompanying nuclear fusion. Concequently, it is assumed that there is a differential metabolic process in both the asexual and sexual growth phase. Thus it seems convenient for studying this mechanism also to examine nourishmental differentiation inducing a qualitative change in the metabolic status of the vegetative and reproductive growth phase. N. Fries[1] determined a nourishmental constitution to be given to the minimal medium which would be favourable to the vegetative growth of this fungus. It contains ammonium tartrate and ammonium nitrate as the nitrogen source in the medium. Westergaard and Mitchell have recommended potassium nitrate (1g./l.) to be the best nitrogen source applied in the synthetic medium to favour the perithecial formation[2]. It became clear that the presence of both ammonium and nitrate is necessary for vegetative growth, and also that the application of nitrate and elimination of ammonium as a nitrogen source in the medium for reproductive growth are required.

Material and Method

The strains used in the present experiment are A(+) and a(−) isolate from the progeny obtained in a crossing of the wild type of *N. crassa* 4A(+) and 8a(−). These strains are normal in every external feature, i. e. in mycelial, macro- and micro-conidial feature. They form fruit bodies after about a 2 week period when cultured on a synthetic basal medium at 25°.

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The medium used for mating in this experiment is the synthetic medium supplemented with various ammonium type nitrogen which contains the same gradients with the synthetic medium* devised by Westergaard and Mitchell[2]. Various ammonium type nitrogen compounds to be added to the synthetic medium are $(NH_4)_2C_4H_4O_6$, $(NH_4)_2C_2O_4H_2O$, $(NH_4)_2SO_4$, $(NH_4)_2CO_3$, NH_4Br, NH_4I, NH_4NO_3 and NH_4Cl. Eight series of agar cultures in which the amounts of these nitrogens varied from 1×10^{-4}M to 1×10^{-2}M to give the ionic concentration ratio of NH_4^+ and NO_3^- varied from 10:10 to 1:10 were set up. As NH_4NO_3 has a nitrate ion group, the amount of KNO_3 contained in the synthetic medium is varied to keep the above ionic concentration ratio. The method devised for studying the effect is as follows: the examination of the direct effect of the minute amount of ammonium is carried out by the perithecial formation test on a slant medium containing various ammonium types of nitrogen in the ionic concentration ratio of NH_4^+ and NO_3^- varied from 10:10 to 1:10. The examination of the indirect effect is done by using a filtrate agent. The process is as follows: A loop of each spore suspension (ca. 28×10^6/ml.) of the A(+), a(−) single strain, and A(+) and a(−) mixed strain is planted in a liquid medium 100 ml. in a flask, and incubated at 26° for 3 to 6 days in a dark chamber. The liquid medium is then filtered through a Seitz Filter (Toyo Asbestos Sterilizing Film No. 85) to remove completely free mycelia and conidia which are measured after drying completly. The culture filtrate or the CF**, contains the filtrate agent. The pH of the medium was adjusted at 6 to 6.5 by Na_2HPO_4 before sterilization. The perithecial formation test as a measure of the effect is conducted by counting the protoperithecia formed in the test tube containing the slant medium.

Experimental results

In order to examine the effect on protoperithecial and perithecial formation of various ionic concentrations of ammonium, a test for the formation and counting of protoperithecia and perithecia was conducted. The influence of the $[NH_4^+]:[NO_3^-]$ ratio in the medium on protoperithecial and perithecial formation was tested in the first experiment. A loop of spore suspension was placed on all synthetic agar media to which had been added various gradient amounts of each of the ammonium type nitrogen sources containing either $(NH_4)_2$ or (NH_4) group. The number of protoperithecia and perithecia formed after 10 to 14 days incubation at 25° in a dark chamber was counted. The result are shown in Table 1. The formation of protoperithecia was normally initiated after a lag period of 5 to 7 days and became stationary within 10 to 14 days after shedding large number of perithecia. Mycelial growth was not so abundant. The formation rates in two cases where either $(NH_4)_2C_4H_4O_6$ or $(NH_4)_2C_2O_4H_2O$ had been added were more remarkable than the

* The gradients are $KNO_3$1g. $KH_2PO_4$1g. $MgSO_47H_2O$ 0.5 g. NaCl 0.1 g. $CaCl_2$ 0.1 g. sucrose 20 g. trace elements, biotin 5×10^{-6} g. agar 20 g. in 1 liter distilled water.

** The term is referred to the culture filtrate by Dr. N. Tanaka (1957).

others. In addition, the number of perithecia increased remarkably at a particular ionic concentration ratio of ammonium and nitrate ions among all ratios tested from 0:10 to 10:10.

In the case of (1) the ratios at which peak of perithecial formation was revealed, were 8:10, 5:10 and 1:10; in the case of (2) they were 9:10 and 6:10; in the case of (3) they were 7:10, 5:10 and 2:10; in the case of (4) they were 8:10, 6:10 and 2:10; in the case of (5) they were 9:10, 6:10 and 1:10; in the case of (6) they were 9:10, 6:10 and 2:10; in the case of (7) they were 9:10, 5:10 and 3:10; in the total they were 8:10, 6:10, and 1:10. At a ratio of 10:10, the case in which the peak appeared was (2); at 9:10, it being in (2), (5), (6) and (7); at 8:10, it being in (1), (4); at 7:10, it being in (3); at 6:10, it being in (2), (4), (5) and (6); at 5: 10, it being in (1), (3) and (7); at 4:10, it being in (2); at 3:10, it being in (1) and (7); at 2:10, it being in (3), (4) (5) and (6); at 1:10, it being in (1), (5) and (6).

Table 1. Perithecial formation resulting from variation in the amounts of ammonium type nitrogen sources.

Ammonium type nitrogen added to KNO_3 / Conc. ratio $[NH_4^+]:[NO_3^-]$	Number of perithecia formed on agar-agar medium supplemented with various concentration ratios of either ammonium or nitrate ion											
	10/10	9/10	8/10	7/10	6/10	5/10	4/10	3/10	2/10	1/10	0/10	Total
1. $(NH_4)_2C_4H_4O_6$	218* ±47**	250 ±22	420 ±17	382 ±22	362 ±23	460 ±36	303 ±15	327 ±30	210 ±23	470 ±27	110 ±23	3512
2. $(NH_4)_2C_2O_4H_2O$	243 ±17	330 ±17	306 ±24	238 ±17	380 ±12	215 ±18	274 ±18	125 ±9	155 ±14	172 ±9	〃	2548
3. $(NH_4)_2SO_4$	40 ±7	60 ±22	140 ±16	173 ±31	145 ±25	190 ±22	167 ±25	144 ±32	182 ±16	118 ±22	〃	1469
4. $(NH_4)_2CO_3$	71 ±8	50 ±8	69 ±14	31 ±5	108 ±22	0	45 ±7	83 ±8	117 ±12	20 ±8	〃	704
5. NH_4Br	26 ±10	200 ±12	137 ±15	72 ±12	156 ±10	80 ±17	44 ±7	92 ±9	130 ±12	140 ±11	〃	1187
6. NH_4I	0	105 ±25	4 ±1	51 ±11	132 ±17	17 ±5	65 ±20	10 ±7	100 ±47	98 ±10	〃	692
7. NH_4NO_3	81 ±28	84 ±17	38 ±8	0	45 ±6	100 ±20	0	105 ±19	0	50 ±11	〃	613
8. NH_4Cl	0	0	0	0	3 ±2	10 ±5	12 ±2	2 ±1	0	0	〃	137
Total	679	1079	1114	947	1331	1072	910	888	894	1067	880	10822

* The average number of ten tubes under culture duration 5–11 days after planting at 26°.

** Standard error $\left(\sqrt{\frac{\Sigma d^2}{n(n-1)}}\right)$.

Furthermore, the second experiment was conducted to investigate the influences of the CFs produced in the synthetic media in which the ratio of $[NH_4^+]:[NO_3^-]$

Table 2. A relationship between various ammonium type nitrogen source combined with potassium nitrate in ten grades of $[NH_4^+]:[NO_3^-]$ and the CFs (±CF, +CF and −CF) obtained from synthetic medium supplemented with these nitrogen sources for protoperithecial and perithecial formation. In the ±CF series A(+) and a(−) were inoculated, in the +CF and −CF series either a(−) or A(+) was inoculated respectively. Ion ratio of $[NH_4^+]$ and $[NO_3^-]$ was varied in the range of 10:10 to 1:10. Measures were accompanied by the standard error.

Nitrogen source \ RATIO	CF agent	Number of protoperithecial formation											
		10/10	9/10	8/10	7/10	6/10	5/10	4/10	3/10	2/10	1/10	Total	Exp. ratio of CF.
1. $(NH_4)_2C_4H_4O_6$	±	28 ±6*	55 ±8	110 ±14	65 ±11	85 ±11	164 ±24	105 ±15	64 ±13	140 ±22	85 ±12	901	1.5:2:1**
	+	7 ±3	23 ±6	58 ±9	58 ±9	92 ±8	240 ±27	183 ±21	118 ±20	133 ±24	165 ±23	1137	
	−	53 ±6	77 ±11	161 ±20	79 ±7	67 ±8	64 ±13	26 ±7	4 ±3	133 ±21	4 ±2	668	
2. $(NH_4)_2C_2H_4H_2O$	±	24 ±6	45 ±14	26 ±16	25 ±5	26 ±4	55 ±12	20 ±9	13 ±4	48 ±13	12 ±3	294	1:1:1***
	+	0	74 ±18	0	53 ±13	14 ±4	79 ±16	—	—	2 ±1	11 ±4	233	
	−	26 ±4	37 ±11	0	40 ±12	40 ±13	23 ±5	29 ±5	45 ±13	16 ±4	15 ±4	271	
3. $(NH_4)_2SO_4$	±	23 ±11	37 ±12	14 ±5	36 ±10	58 ±16	41 ±6	45 ±5	24 ±6	69 ±17	17 ±5	364	1:1:1***
	+	85 ±11	48 ±15	2 ±2	0	56 ±14	36 ±11	0	1 ±1	41 ±12	64 ±15	333	
	−	119 ±18	40 ±11	46 ±12	13 ±4	46 ±11	72 ±11	0	16 ±6	12 ±4	10 ±1	374	
4. $(NH_4)_2CO_3$	±	0	0	0	0	0	0	10 ±1	0	0	0	10	0.5:11:1
	+	18 ±11	16 ±11	15 ±7	2 ±1	38 ±12	11 ±9	18 ±8	70 ±12	33 ±9	21 ±8	242	
	−	0	0	0	3 ±1	6 ±3	12 ±7	4 ±3	0	0	0	22	
5. NH_4Br	±	89 ±7	109 ±6	88 ±7	105 ±5	99 ±4	45 ±4	59 ±3	58 ±5	85 ±6	50 ±4	787	2:1
	+	84 ±6	34 ±5	84 ±8	8 ±3	5 ±2	30 ±3	96 ±6	18 ±4	6 ±2	12 ±3	377	
	−	0	0	0	0	0	0	0	0	0	0	0	
6. NH_4I	±	0	0	1	3 ±1	3 ±1	5 ±2	7 ±3	7 ±2	3 ±1	3 ±1	31	0.5:0.5:1
	+	2 ±1	5 ±2	0	0	10 ±4	3 ±1	1 ±1	0	0	6 ±2	27	
	−	0	0	0	0	13 ±4	0	31 ±5	0	17 ±3	8 ±3	69	
7. NH_4NO_3	±	29 ±6	34 ±2	13 ±3	33 ±4	6 ±4	26 ±5	42 ±6	22 ±3	0	3 ±2	208	1:1:1
	+	30 ±12	15 ± 3	46 ±14	16 ±3	16 ±3	0	16 ±2	0	0	8 ±2	137	
	−	26 ±7	41 ±6	60 ±9	3 ±2	18 ±3	0	24 ±4	1	7 ±2	11 ±3	191	
8. NH_4Cl	±	54 ±9	72 ±12	70 ±13	85 ±14	50 ±7	60 ±9	35 ±6	36 ±6	32 ±7	40 ±7	534	3:1.4:1
	+	16 ±5	66 ±11	50 ±8	45 ±9	24 ±7	18 ±4	16 ±6	16 ±6	6 ±3	0	257	
	−	35 ±6	17 ±4	12 ±3	19 ±4	30 ±6	20 ±6	7 ±3	9 ±4	7 ±3	30 ±3	186	

* Standard error

** Highly significant χ^2 ($P<0.01$)

*** Significant χ^2 ($0.05<P<0.2$).

was changed in 10 grades for every combined ammonium salt. The protoperithecial formation test was conducted by inoculating two sexual mating types, A(+) and a(−), on the agar medium supplemented with either the +CF or −CF prepared from the liquid synthetic media in which single culture of two mating type hyphae was grown. The results were shown in Table 2. In general, in the case of (1), (2), (3), (7) and (8) the effective formation was revealed. The concentration ratio of the ions in question in the medium was certainly related to the perithecial yield. In order to see whether the significant difference does exist or not in the experimental lots for perithecial yield, the standard error $\left(\sqrt{\frac{\Sigma d^2}{n(n-1)}}\right)$ was caluculated and described in Table 2 as well as in Fig. 1. In the first experimental case (1) the ratios at which the peak of perithecial formation was revealed, were 8:10, 5:10 and 2:10 in the ±CF series; they were 5:10 and 1:10 in the +CF series; they were 8:10 and 2:10 in the −CF series. As a whole, the developmental stimulation in the experimental lots (1), (2) and (3) supplemented with the ±CF was seemed to be intermediate of those observed in the +CF and −CF series, and in others it was predominant.

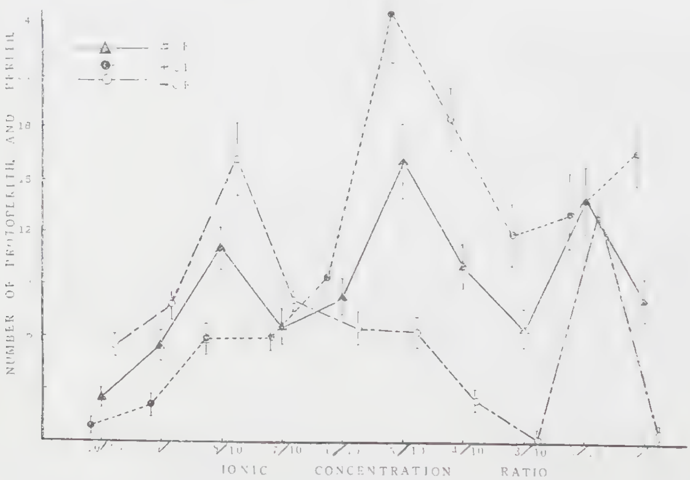

Fig. 1. The influence of CFs from the synthetic medium containing ammonium and nitrate in 10 grades ionic concentration ratio on the protoperithecial and perithecial formation. The signs used in the figure show the following notes:

——▲——: perithecial formation of A(+) and a(−) by ±CF,
------●------: protoperithecial formation of a(−) by +CF,
-−-−○-−-−: protoperithecial formation of A(+) by −CF,
——I——: range of standard error.

The numerals in abscissa indicate the ratio of $[NH_4^+]:[NO_3^-]$ in the synthetic media for preparation of the CF.

Consideration

In the sexual reproductive process, the physiological sequence concerned with

the fruit body formation in bipolar fungi, *N. crassa*, should be pursued primarily by analyzing conditions which seem to be responsible for creating differentiation in the physiological process which may work in developing a reproductive phase from a vegetative one. This will partly be achieved through an examination of the nourishmental differentiation among the minimal media and of a lot of synthetic media that were recommended by several investigators in the past. The minimal medium used heretofore for the vegetative growth of *N. crassa*, contains $(NH_4)_2C_4H_4O_6$ 5 g. ($5/2\times10^{-2}$M) and NH_4NO_3 1 g. ($4/3.2\times10^{-2}$M) as the nitrogen sources[2]. Meanwhile the synthetic medium recommended for the reproductive growth contains only KNO_3 1 g. (1×10^{-2}M) without ammonium compound[2]. In the minimal medium, if all nitrogen sources had dissociated completely into the ionic condition in the medium, the ionic concentration ratio of $[NH_4^+]:[NO_3^-]$ should be $5+4/3.2:4/3.2=5:1$, and in the latter this ratio will be 0:1. This fact, namely, seems to indicate that the excess amount of NH_4^+ in the medium may be responsible for reduction in the perithecial formation in reproductive growth. At the same time, less frequent formation of the perithecia to be produced when this fungus cultured in the synthetic medium which contains only NO_3^- as a nitrogen source might be due to lack of NH_4^+ in the medium. It should be expected, at the same time, that the activity of NH_4^+ in the synthetic medium might have an analogous inhibiting activity for perithecial growth as in the case when the amount of some metal ion is too great.

On the activity of NO_3^- in the perithecial formation Hirsh[3] stated that protoperithecial formation increased with an increasing of the amount of KNO_3 until it reaches a maximum at a concentration of 1 g. per litre after which the number fell off. Furthermore, he examined precisely the nitrate effect and concluded that the presence of NO_3^- did not affect fertilization and fruit body development but rather some stage in the differentiation of the protoperithecium, and that there should be a radically differential mode of utilization of nitrate in conjunction with the appearance of the two different "physiological states" at two temperatures.

As shown in Table 1, the distribution frequency in the number of perithecia is not randomised but shows clotting somewhat; roughly speaking, around the three different ionic concentration ratios of $[NH_4^+]$ and $[NO_3^-]$ 8:10, 6:10 and 1:10. It is presumably considered that there are three peaks represented by the three concentration ratios of $[NH_4^+]$ and $[NO_3^-]$. It may possibly indicate the presence of a specific feature in the utilization of a minute amount of both NH_4^+ and NO_3^- for perithecial formation by two sexual mating type strains. This may depend also upon the differential utilization of both NH_4^+ and NO_3^- based upon the physiological differentiation of either sexual mating type strains, because, as shown in Fig. 2 in the previous report[4], in the column 1, 2 and 3 in the Table 2 and Fig. 1, either the +CF or −CF effectively acts on perithecial formation for the opposite sexual mating type strain respectively, especially, at two definite ionic concentration ratio of NH_4^+ and NO_3^-. The +CF has a stimulative activity for protoperithecial formation at the

ionic concentration ratios $[NH_4^+]:[NO_3^-]=5:10$ and $1:10$, on the other hand the −CF at 8:10 and 2:10. That is, it is assumed that A(+) strain would produce more stimulative substances at certain lower concentration of NH_4^+ than the a(−) strain does, and at certain higher concentration of NH_4^+ *vice versa*. The ionic concentration ratios $[NH_4^+]:[NO_3^-]$ at which either the +CF or −CF reveals discriminative effects on protoperithecial formation are different from each other. As shown in Table 2 (column 1, 2 and 3) and Fig. 1, either A(+) or a(−) produces more markedly stimulative substance at two concentration ratios, viz. 5:10 and 1:10, 8:10 and 2:10, of NH_4^+ and NO_3^- on the protoperithecial formation. Thus, the remarkable perithecial formation, as shown in Table 1, at ratios 8:10, 5:10 and 1:10 in the case of (1), at ratios 9:10, 6:10 and 4:10 in the case of (2) and at ratios 7:10, 5:10 and 2:10 in the case of (3) etc. could be considered as a combined effect of the +CF and −CF on the perithecial formation which is initiated by protoperithecial formation of single sexual strain mycelia occured by stimulative substances which are produced abundantly at a particular ratio of these ions in the CFs produced from the complementary strain culture. Furthermore, this interpretation could be justified by comparing the perithecial yields in the ±CF series with those in the +CF series and −CF series in the Table 2. If the formation effect of the ±CF is a sum of that of both the +CF and −CF as presumed in the previous report[5], that is;

$$\begin{matrix}\text{The formation effect}\\ \text{of the } \pm\text{CF}\end{matrix} = \begin{matrix}\text{The formation effect}\\ \text{of the } +\text{CF}\end{matrix} + \begin{matrix}\text{The formation effect}\\ \text{of the } -\text{CF.}\end{matrix}$$

the perithecial yield in the ±CF should be one half of the sum of the yield in the +CF and −CF when approximately equal number of spore were inoculated. In the case of (1), (2), (3) and (7) in the Table 2, the ratios of the formation yield among the three CF series were approximately estimated 1.5:2:1, 1:1:1, 1:1:1 and 1:1:1, respectively. However, in the case of (8) it was 3:1.4:1. Thus, it can be concluded that the formation effect of the ±CF series seen at the three concentration ratios would be composed of (a) the effect of the +CF and (b) that of the −CF as shown in the case of the (1), (2), (3) and (7), and of the either effect and the effect of unknown factor in the case of the (8).

The problem of the stimulative substances in *Neurospora* is interesting to be solved concerning the nature of the mating type factor and the biochemical mechanisms in the sexual reproduction in this organism as well as in the other fungi. E. R. Sansome[6] also stated that although the sexual reproduction depends upon the fusion of two nuclei of opposite mating types, there is no evidence that heterokaryon formation is necessarily required for perithecial formation, and it would be caused by the stimulation of the mycelium of one mating type by hormone-like substance produced by the other mating type. In this experiment the existence of the sexual hormone-like substances has been clearly recognized as well as in the other species.

If the developmental perithecial formation seen at the several ionic concentration

ratio is initiated by several sexual hormone-like substances, it seems that either sexual mating type factors (mt^+ and mt^-) act to produce these substances at these ionic concentration ratios and they work independently to produce at least two kinds of the stimulative substances. At the same time, it seems to be likely that the production process of the substance by the both strains is composed of at least two processes in relating to nitrate reductase system. This explanation described above is supported by the following facts reported by Hirsh[3] and W. S. Silver and W. D. McElroy[7].

According to Hirsh, in *Neurospora crassa*, a lot of protoperithecia and perithecia were formed only when the organism was grown at 25° and kept continuously at the same temperature. When the mold was grown first at 35° and transferred to 25° after conidiation produced also a lot of mature perithecia. While those cultured continuously at 30° produced incipient protoperithecia which do not develop further. However, when the organism was grown at 35° and kept on at the same temperature after conidiation, neither protoperithecia nor perithecia were formed. When the culture was kept at 25° before conidiation and transferred to 35° after conidiation, only protoperithecia were formed, but they did not develop into mature perithecia. Thus, it was considered that some stimulative substances were produced at the temperature range between 25° and 35° before conidiation. But this prediction was not proved substancially, for the extract of any culture before conidiation exerted neither stimulative effect nor inhibitory effect for fructification in the culture after conidiation.

On the other hand, W. S. Silver and W. D. McElroy showed that there were several enzymatic steps in the reduction process of nitrate to nitrite and they were controlled by several different genetic factors which affected either directly or indirectly the synthesis of nitrate reductase.

Consequently, it is suggested that the induction and initiation of the perithecial and protoperithecial formation will be presented differentially by trace agents contained in the filtrate agent of either the +CF or −CF, in other words, the whole sexual reproduction will be consisted at least of two different physiological processes of either the A(+) or a(−) differentiated in the utilization of both ammonium and nitrate. In order to make evident this problem, it seems necessary that (i) to analyze quantitatively the amount of turnover of the NH_4^+ and NO_3^- with the lapse of culture time under some temperature range, and that (ii) to demonstrate chemically as well as physically the potential activity of the specific substances in the CFs for the protoperithecial formation.

The author wishes to express his many thanks to Dr. N. Tanaka for helpful and valuable suggestion to describe this report and to Dr. J. R. Raper for his encouragement in the achievement of the work.

Summary

The present report deals with the effect of different ammonium type nitrogens in gradient amounts on the perithecial formation in *Neurospora crassa.*

The perithecial induction is activated in media containing either ammonium tartrate or ammonium oxalate rather than the other ammonium type compounds as nitrogen source. It is made evident that the perithecial formation is conspicuously activated in the media supplemented with both potassium nitrate and ammonium tartrate to make up the ionic concentration ratio of ammonium and nitrate to be either 8:10, 6:10 or 1:10 (cf. Table 1).

This activity is found to be related to some substance produced in the media under special ionic conditions; the filtrate of the A(+) culture (+CF) produces substance which stimulates the perithecial formation of the opposite a(−) mycelia and the filtrate of the a (−) culture (−CF) produces substance that activates the A(+) perithecial formation.

There is a compensating declination in the effect of the single filtrate on the perithecial formation; in the A(+) strain culture, the stimulative substance increases in the lower ammonium concentrations and in the a(−) strain culture it increases in the higher. At the same time, the filtrate of the mixed culture (±CF) has a combined effect of both single filtrate (+CF and −CF) on the perithecial formation.

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摘　　要

本報は *Neurospora crassa* の被子器形成におよぼす種々のアムモニウム態窒素源の量的影響についての研究報告である。被子器形成は，用いられた種々のアムモニウム態窒素源のうち，酒石酸アムモニウムを微量(0.5×10^{-3}M 以下) 添加の合成子実体形成用培養基上でいちじるしい。特に前者の場合 NH_4^+ と NO_3^- の濃度比が8:10, 6:10 および 1:10 の時に顕著である。

この添加窒素源の特定量添加の際にみられる形成作用は，NH_4^+ と NO_3^- の特定濃度比を有する合成培養基中で，A(+) 系および a(−) 系がそれぞれその反対性型系すなわち a(−) 系および A(+) 系菌糸に原被子器 (protoperithecium) 形成を誘起する性ホルモン様物質のはたらきによることが明らかとなった。そしてA(+) 系は NH_4^+ 濃度が低い場合，a(−) 系は NH_4^+ 濃度が高い場合に著るしい原被子器形成の誘起作用をあらわし，A(+) および a(−) 混合系では両者の中間的効果がみられる。

An Analysis of Sprout Growth after Felling in Cultivated Mulberry Plants

by Tadayoshi TAZAKI*

田崎忠良*： 栽培クワにおける伐採後の生長解析

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As was mentioned in a previous report[1], the shoots of cultivated mulberry are once cut down annually in early spring or early summer. Felling in the growing season may give serious influences on the physiology of woody plants. After exhausting reserve substances for vernal growth the assimilatory organs with stem are entirely lost by felling, so the growth materials for new sprout, at least in the earliest stage, must depend upon remaining reserve substances and may be gradually replaced by the assimilates of new sprout. The indirect proof of this process can be given by the variation of reserve substances in stump and subterranean part as in the research of Taguchi and Nishimura[2], of "spring cut" and "summer cut" mulberry. The author also attempted to follow the seasonal variation of reserve starch by microchemical method and ascertained the disappearance of reserve starch in stem and root in early summer, before and after summer cutting. The quantitative estimation of the difference in hot-water soluble carbohydrates (mostly sugars and starch) just before and after summer cutting failed on account of the fluctuation of individual plants.

In the present report, the nature of sprout growth after felling was analysed from the relationships between gross production and distribution of assimilates. This line of study was commenced by Boysen-Jensen[3] and was developed by Monsi and Hôgetsu groups in our country[4),5]. Recently, Iwaki[6] analysed the density effect in *Fagopyrum esculentum* community from this view point.

Material and method

The materials for this experiment were the cultivated mulberry (Kairyo-nezumi-gaeshi, a form of *Morus alba*) in the mulberry field of the Tokyo University of Agriculture and Technology situated in Koganei-shi, Tokyo. The details of culture mode were already mentioned in a previous paper[1]. In order to follow the growth process in "summer cut" mulberry, some of the shoots were cut down biweekly and the dry weight of leaves and stems were measured after drying in an electric drying oven at 105°. The shoot length of all shoots of the mulberry field (1 are) was

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measured, after which five shoots with average shoot length were cut down for the dry weight measurement. The measurement was duplicated in 1953 and 1954 with similar results. As the check the growth process of seedlings (*Morus Lhou*) sown on June 18 was followed. Seeds were sown by broadcast sowing on a flat seed bed with adequate chemicals and barnyard manure. After germination, seedlings were thinned in the early stage. The distance of each plant was around 5 cm. The sampling method was similar to that of "summer cut" mulberry. The measurement was done about weekly by which the sample seedlings were dug up as perfectly as possible and the dry weight of roots together with those of leaves and stems was determined. The measurement was also duplicated in 1954 and 1956.

The dry weight of leaves, stems and roots (in the case of seedlings) per one shoot (plant) was converted to carbon amount by the carbon percentage of the dry matter estimated by Tiurin's titration method[7]. The accumulative gross production (mg. carbon per one shoot or plant) was calculated from the leaf amount and daily gross production (mg. carbon per mg. leaf dry weight).

Result and discussion

First of all, the growth process of seedlings will be analysed. The measurement was commenced on July 2, 1956 and finished on November 19 of that year. From July 14 to September 1, the growth process of each plant part was hemilogarithmically linear (Fig. 1-A), so the empirical formula

$$A=A_0e^{kt}$$

was adapted, in which A_0 and A are the dry weight of each plant part at the beginning and after t days respectively and k is a constant. The formulae for leaves (F), stems (C_h) and roots (C_w) were

$$F=1.00e^{0.1172t} \tag{1}$$

$$C_h=0.24e^{0.1206t} \tag{2}$$

$$C_w=0.30e^{0.1162t} \tag{3}$$

The carbon content of leaves, stems and roots was 41, 45 and 44 % in oven dry basis, respectively. The dry weight calculated from above formulae was converted to carbon content and additively illustrated in Fig. 2-A.

For the calculation of accumulative gross production (ΣP), daily gross production was integrated for the given interval, that is, from t_1th to t_2th day, thus

$$\Sigma P=\int_{t_1}^{t_2} pF_0e^{kt}dt=p\frac{F_0}{k}(e^{kt_2}-e^{kt_1})$$

$$=p\frac{1}{0.1172}(e^{0.1172t_2}-e^{0.1172\times 12}) \tag{4}$$

in which p is the daily gross production of unit amount of leaf in carbon, and t_1 was 12 in this case. F_0 is the initial amount of leaves.

The value of p was estimated from, (1) the daily march of illumination in Tokyo and her vicinity for fine days during July and August[6),8)], (2) the net assimi-

lation amount of mature mulberry leaves under light saturated condition[1], (3) the nocturnal respiration of mature leaf and (4) the dry weight of unit area of leaves in order to convert the net assimilation amount from area to a dry weight basis.

For the calculation of p, the duration of photosynthetic activity was estimated as 11 hours, nocturnal respiration as 13 hours with the rate of 0.5 mg. CO_2/50 cm.^{2}/hr. The mean dry weight of 1 cm.^{2} of leaf was 3.5 mg. The values of p when net assimilation is 3, 4, 5 and 6 mg. CO_2/50 cm.^{2}/hr. are as shown in Table 1.

Substituting these p values in formula (4) the accumulative gross production for each value of net assimilation can be obtained. The time course of the accumulative gross production was also illustrated by broken lines in Fig. 2-A.

Lastly, the accumulative respiration amount of stem and root was calculated from similar formulae as formula (4), putting the respiration amount in carbon for unit dry weight (r_c) in place of p and initial stem and root amount (C_{h0}, C_{w0}) in place of F_0. The measurement of r_c was not done in seedlings, and the value was adapted from the measurement of mature mulberry, which was 100 mg. CO_2/50 g. dry weight/hr. Here 50 g. dry weight roughly corresponds to 100 g. of fresh weight. This respiration value may be somewhat larger than the actual value when compared with the data of Iwaki in *Fagopyrum esculentum*[6]. The time course of accumulative respiration was also illustrated in Fig. 2-A in addition to F, C_h and C_w.

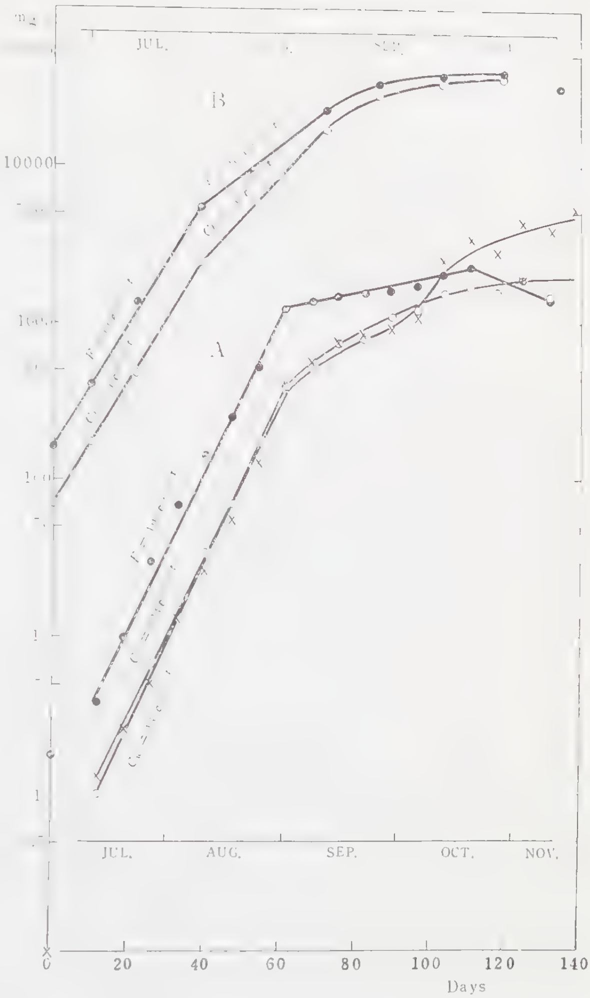

Fig. 1. Dry weight (per plant) of leaf (F), stem (C_h) and root (C_w) in the growth process of mulberry yearlings (A), and that of leaf and stem (per shoot) in the growth process of "summer cut" mulberries (B). Seeds were sown on June 18, 1956 in yearlings, and shoots of the previous year were cut down on May 21, 1954 in "summer cut" mulberries. In both cases petioles were included in stem.

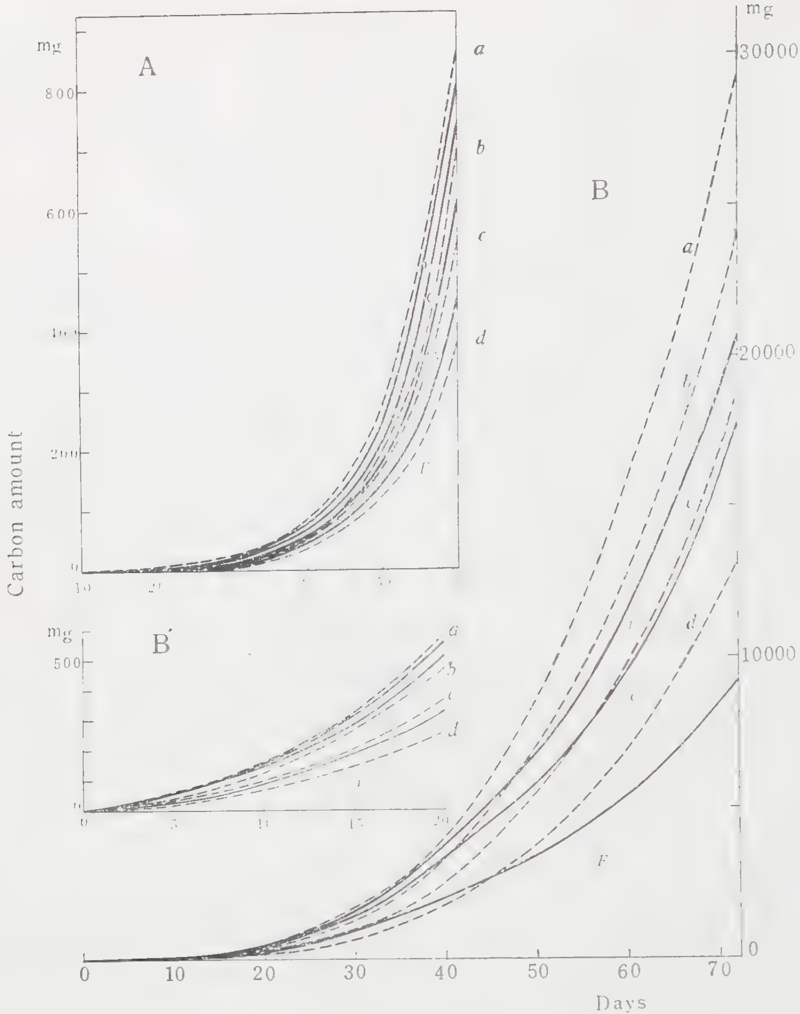

Fig. 2. Growth process of each plant part and accumulative gross production in the seedlings (A) and "summer cut" mulberry (B). In the case of "summer cut" mulberry the initial process was enlarged (B)′. Accumulative gross assimilation curves of a, b, c and d are based on 11 hours of assimilation with the rate of 6, 5, 4 and 3 mg. CO_2/50 cm².hr. The symbols F, C_h, C_w and r_c are the carbon amount of leaf, stem and root, and the accumulative respiration of non-assimilating organs.

Table 1. The values of gross production per unit amount of leaf (p) calculated from several net assimilation values (Seedlings)

a. Net assimilation (mg. CO_2/50 cm.²/hr.)	3	4	5	6
*b. Gross production (mg. CO_2/50 cm.²/day)	26.5	37.5	48.5	59.5
**c. Gross production (10^{-2} mg. C/mg. dry weight/day)	4.13	5.85	7.56	9.27

* $b = a \times 11 - 0.5 \times 13$, ** $c = b \times \frac{1}{175} \times \frac{12}{44}$

In Fig. 2-A the production side is the accumulative gross production and the distribution side consists of $F+C_h+C_w+r_c$. By this figure, 11 hours of assimilation with the rate of 6 mg. CO_2/50 cm.^{2}/hr. or thereabout can cover the distribution side. The net assimilation amount of most leaves of seedling in summer, likewise "summer cut" mulberry, attained 6-8 mg. CO_2/50 cm.^{2}/hr. under light saturated condition. Taking into account the weather condition, low activity of younger leaves, "afternoon nap" and mutual shading of lower leaves, the actual net assimilation amount in field condition may be somewhat diminished. So the supposed amount of 6 mg. CO_2/50 cm.^{2}/hr. or thereabout from the growth analysis of seedlings may be adequate.

In the light of growth process in seedlings, let us turn to the same process in "summer cut" mulberry. The data of 1954 was used for calculation. Shoots of the previous year were cut down on May 21. The shoot growth from June 21 to September 2 was analysed. The linear relation between the time and the logarithm of dry weight of shoot and leaf has a bending point at July 30, so the straight line was divided into two parts (Fig. 1-B), thus for leaf

from June 21 to July 30 $\quad F=160e^{0.0902t}$ (5)

from July 30 to Sept. 1 $\quad F=1000e^{0.0432t}$ (6)

and for stem

from June 21 to July 30 $\quad C_h=70e^{0.0949t}$ (7)

from July 30 to Sept. 1 $\quad C_h=300e^{0.0575t}$ (8)

Likewise seedlings, the dry weight of leaves and stem was converted to carbon amount and additively illustrated in Fig. 2-B. The growth process of roots was not measured. The seasonal and partial variation of stem respiration was measured in our laboratory in 1957.* A part of stem was cut and the cut ends and the base of petioles after detaching leaves were immediately sealed with sealing wax. The length of stem parts was chosen as long as possible for the measurement in order to minimize the influences of cut end. By this consideration, the stem length was in most cases 40 cm., but in some unavoidable cases the length was somewhat shortened. The stem parts were then put in black coated glass tubes and immersed in a thermostat kept at 20°. The carbon dioxide evolved out of stems was measured by Boysen-Jensen's apparatus. By continuous measurement the value steadily decreased and attained a constant value at 5 hours after detachment, and this value was regarded as actual respiration amount. The values in autumn and early spring conformed well with that of *Fraxinus* measured by Müller[10], that is, 5-8 mg. CO_2/50 g. dry weight/hr. In summer, remarkable variation was observed. The case of "summer cut" mulberry was illustrated in Fig. 3. The values on the whole were far larger than those of early spring and autumn, and rapidly decreased as the

* Unpublished data. A part of the summary of this study was printed in the following literature. Honma, S. and Tazaki, T., Jour. of Sericultural Sci. of Japan **26** (3): 204, 1957.

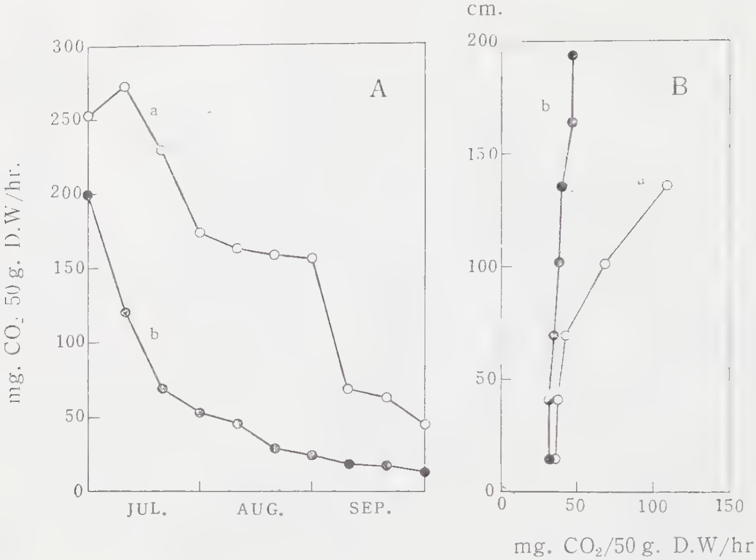

Fig. 3. Seasonal and partial variation of stem respiration. (A) Seasonal variation of stem respiration in "summer cut" mulberry. a. upper part and b. lower part. (B) Partial variation of stem respiration on Aug. 23, 1957. a. "summer cut" shoot and b. "spring cut" shoot,

aging of the stem. So the values in upper young part were always larger than the lower old part. Considering the variations of respiration amount and the temperature in summer, the value of 100 mg. CO_2/50 g. D.W./hr. was assigned for the calculation of accumulative respiration of stem from July to August. The accumulative respiration was calculated likewise seedlings and was additively illustrated in Fig. 2-B. So far the distribution side was completed except the carbon marterials used for the respiration and growth of roots and stump, for which it will be referred afterwards.

For the calculation of production side the daily gross production of unit amount of leaf (p) was calculated at first. The basis for the calculation was the same as seedlings except the dry weight of leaf which was 5 mg./cm.^{2} in "summer cut" mulberry. The values of p in different rate of net assimilation are as shown in Table 2.

Table 2. The values of gross production per unit amount of leaf (p) calculated from several net assimilation values ("summer cut" mulberry)

a. Net assimilation (mg. CO_2/50 cm.^{2}/hr.)	3	4	5	6
*b. Gross production (mg. CO_2/50 cm.^{2}/day)	26.5	37.5	48.5	59.5
**c. Gross production (10^{-2} mg. C/mg. dry weight/day)	2.89	4.09	5.28	6.49

* $b=a\times 11-0.5\times 13$. ** $c=b\times\frac{1}{250}\times\frac{12}{44}$

By the same procedure as seedlings the accumulative gross production for each value of net assimilation amount was calculated and was illustrated by broken lines in Fig. 2-B. In this figure, 11 hours of assimilation with the rate of 6 mg. CO_2/

50 cm.^{2}/hr. can scarcely cover the distribution side during July and the rate of 5 mg. CO_2 falls below the distribution side. Considering the various factors of diminishing net assimilation mentioned above, such a high rate as 6 mg. CO_2 can hardly be realized in field conditions. Besides, it must be remembered that the respiration and growth of roots and stump were omitted in the distribution side. To date we have unfortunately no reliable data on the quantitative growth process in cultivated mulberry despite the long course of the study of mulberry culture. Only Takagi[10] measured by "cylinder method" the total fresh weight of cultivated mulberry root (slender roots with diameter below 1 mm. were omitted), being 7 kg. in a 10-year-old cultivated mulberry (Kairyo-nezumigaeshi). The date of measurement was not written in his paper, but by the photograph therein the season is conjectured to be summer. Assuming the water content to be 270 % on oven dry basis, this root fresh weight corresponds to 1.9 kg. dry weight. Also the standing crop of the above ground part in another 10-year-old "summer cut" mulberry was measured on September 21, 1953 by the author. The fresh weight of leaves, stems and stump was 2.35, 2.84 and 5.63 kg. per plant, respectively. Assuming the water content of leaves, stems and stump to be 250, 250 and 100 %, these fresh weights correspond to 0.67, 1.13 and 2.81 kg. By above mentioned two measurements the dry weight of stump+root in a 10-year-old mulberry is estimated to be 4.7 kg. The dry weight of stump+root at the planting of seedlings is commonly 0.3 kg., so the growth in 10 years is 4.7-0.3=4.4 kg. Under the assumption of linear growth, one year's increment is 0.44 kg. or 48 g. per one shoot (9 shoots in one plant). This amount is the same order with the growth of leaves and stem in Fig. 1. Taking into account the above mentioned considerations it may be concluded that at least in the earlier stage of shoot growth after "summer cutting" the most efficient matter production as 6 mg. CO_2/50 cm^2/hr. can not cover the growth and respiration of non-assimilating organs and so the carbon materials required for this process must depend upon reserve substances. Besides, considerable amount of leaves and new side branches develop before "summer cutting" from the shoots of the previous year. The leaf amount developed in this season was 55 g. dry weight for one shoot. As mentioned in a previous report[1] the photosynthetic activity before "summer cutting" was comparatively low, so the dependence upon reserve substances is also indispensable for the growth before "summer cutting." After exhausting reserve substances for the spring growth, the shoots are cut down and the new shoot growth afterwards again requires reserve substances, which must be harmful for the physiology of mulberry plant.

In the later stage of growth (from the beginning of August), the accumulative gross production remarkably increased and there arose the possibility of gross production to cover the distribution side. So the relation between production and distribution side is quite different from seedling. After the middle of September the growth of shoot stops (Fig. 1) and the assimilates may be reserved for the

growth of the next year.

Summary

The sprout growth of "summer cut" mulberry after felling was analysed from the relationships between the gross production and distribution of assimilates. The growth process of seedlings sown in early summer was also analysed as the check. The distribution side consists of the growth of leaves, stems, roots and the respiration of these plant parts except leaves. The growth was measured directly and the respiration was calculated from the CO_2 evolution of cut shoots. The production side is the accumulative gross production calculated from net assimilation and nocturnal respiration.

In the summer growth of seedlings, 11 hours of net assimilation with the rate of 6 mg. CO_2/50 cm.^{2}/hr. can cover the distribution side, but in the shoot growth of cultivated mulberry after "summer cutting," even such an efficient net assimilation as 6 mg. CO_2/50 cm.^{2}/hr. can hardly cover the distribution side at least in the early stage of growth. In the later stage the accumulative gross production increased and there arose the possibility to cover the distribution side. High growth in comparison to gross production in early stage is the proof of dependence upon reserve substances.

The author wishes to express his sincere thanks to Prof. M. Monsi and Prof. K. Hôgetsu for their valuable advice throughout the progress of this study and thanks are also due to Messrs. B. Akiyama and T. Tamari who helped the experiments.

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摘　　　要

栽培クワにおける"夏切り"後の生長を，総生産量と同化生産物の分配の面から解析した。また夏のはじめにまいた実生苗の生長経過も対照として解析した。分配側は葉・茎・根の生長と茎・根の呼吸からなっている。生長は直接測定し，呼吸は切りとった茎からの炭酸ガス発生量から計算した。生産側は総生産量の積算値であり，葉の純同化量と夜の呼吸量から計算した。実生苗の夏の生長では，同化時間を 11 時間として純同化量 6 mg. CO_2/50 cm.^{2}/hr. で分配側を満たすことができる。ところが"夏切り"後の栽培クワの生長では，6 mg. CO_2/50 cm.^{2}/hr. という高い能率の純同化量でも，少なくとも初期の生長は満たすことはできない。生長の後期には積算総生産量がふえ分配側を満たす可能性がでてくる。初期において総生産量に比較して生産量が大きいことは，この時期においては貯蔵物質を使っていることを示している。

Ecological and Physiological Studies on the Vegetation of Mt. Shimagare

II. On the Crescent-shaped "Dead Trees Strips" in the Yatsugatake and the Chichibu Mountains**

by Hideo IWAKI* and Tsumugu TOTSUKA*

岩城英夫*・戸塚績*: 縞枯山の植生についての生態学的ならびに
生理学的研究 II. 八ヶ岳・秩父の半月形縞枯について

Received March 10, 1959

In the first work of this study, Oshima *et al*[1] (1958) have made a preliminary survey of the "dead trees strips (Shimagare)" of Mt. Shimagare (2395 m. above sea level) in Nagano Pref., central Japan, and obtained much information on the structure of a "Shimagare" type of forest. Further survey revealed that the occurrence of the dead trees strips was not confined in Mt. Shimagare but a large number of similar phenomena, though of a small scale, were found in the northern part of the Yatsugatake Mountains and in the Chichibu Mountains (Fig. 1), and that many of these dead trees strips had the shape of a crescent. Detailed investigation in the structure and the occurrence in these regions of such dead trees strips will be useful for the explanation of the origin of the "Shimagare" type of forest in Mt. Shimagare, and the present study was conducted on this line.

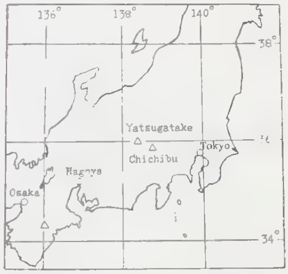

Fig. 1. Map showing the geographical location of the Yatsugatake and the Chichibu Mountains in central Japan.

Dead trees strips in the North-Yatsugatake Mountains

The map of Fig. 2 shows the locations of dead trees strips in the North-Yatsugatake Mountains. On the south-west slope (17°–24°) of Mt. Naka (2493 m.), there can be seen many dead trees strips of smaller scale, one of which showing three whitish stripes with two Forest Units (cf. also Oshima *et al*[1]). A large number of dead trees strips are also found on the south-west slope (24°–31°) of Mt. Tateshina (2530 m.) and in other mountains. The slopes of these mountains are covered with

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Fig. 2. Map showing the locations of dead trees strips in the northern part of the Yatsugatake mountain groups.

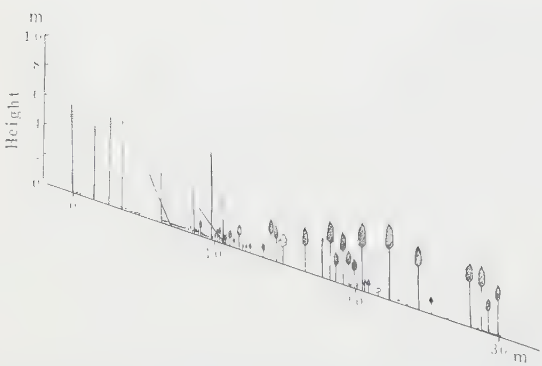


Fig. 3. Profile chart of the vegetation at a crescent-shaped dead trees strip (2420 m. above sea level) in Mt. Yokodake.

the subalpine coniferous forest, similar in Mt. Shimagare, and most of dead trees in these areas are *Abies Veitchii* and *A. Mariesii.*

Many of these small dead trees strips have a characteristic shape of "crescent" of different sizes, and sometimes two or three of them combine into one forming a wavelike strips. In order to investigate the structure of it, a line transect of about 30 m. long was laid down along the south-west slope of Mt. Yokodake (2472 m.) across the middle of one of the crescent-shaped dead trees strips (ca. 2420 m. above the sea), of which convex part projects upwards, i. e., to the north-eastward. Height and diameter breast high of all trees included in the transect were measured, and the results of this measurement are illustrated in a profile chart of Fig. 3. At the dead trees strip, of which width is ca. 10 m., there exist a great number of growing seedlings of *Abies* tree. Descending the slope, these young growths increase their heights until their maxima of about 5 m. are reached at 20 m. downward from the upper end of the dead trees strip. This structure of the vegetation indicates that the dead trees strip will shift upwards every year. The marked similarity of this profile chart to those of Mt. Shimagare (see the previous paper, Fig. 2) suggests that the crescent strips may be the earlier stages of development of the long, conspicuous dead trees strip like those in Mt. Shimagare. Further discussion about this problem will be given later in the present paper.

Dead trees strips in the Chichibu Mountains

The preliminary survey conducted, on the advice of Mr. S. Ichimura, in autumn of 1958 in the north-western part of the Chichibu Mountains has shown a rich existence of the dead trees strips in Mt. Sanpô (2483 m.), Mt. Kobushi (2470 m.), Mt. Tokusa (2469 m.) and in Mt. Karisaka (2289 m.) (Fig. 4 and Table 1.). The most prominent sight can be seen on the south-east slope (26°–38°) of Mt. Tokusa, where two or three whitish stripes develop with many crescent shapes of dead trees strips. Characteristic shapes of the crescent strips are also found along the mountain ridge on the south slope (25°–36°) of Mt. Karisaka. Taking a wide survey in this mountain area, perhaps we shall find another dead trees strips in good numbers in other mountains than these mentioned above.

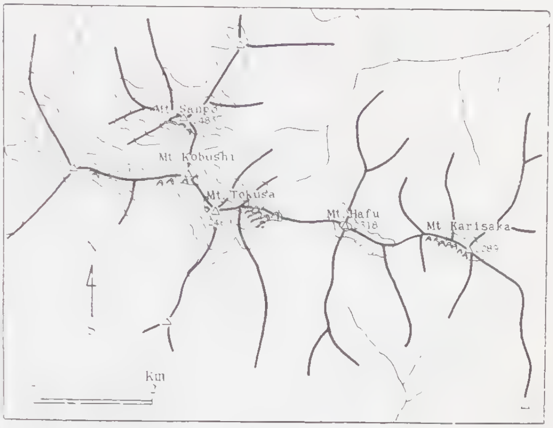


Fig. 4. Map showing the locations of dead trees strips in the north-western part of the Chichibu Mountains.

Table 1. Dead trees strips in the northern part of the Yatsugatake and the Chichibu Mountains.

		Main Tree Species	Altitude (m.)	Slope
Yatsugatake	Mt. Tateshina A.	*Abies Veitchii* and *A. Mariesii*	2300-2450	S-SE (17°-37)
	〃 B.	〃	2100-2380	S-SW (24 -31°)
	Mt. Yokodake	〃	2150-2430	SW (17°-32°)
	Mt. Amaike	〃	2250-2320	S-SW (10 -28)
	Mt. Shimagare	〃	2250-2395	SW (13 -19)
	Mt. Naka	〃	2350-2480	S-SW (17°-24°)
	Mt. Inako	〃	2300-2380	W (14°-20°)
Chichibu	Mt. Sanpô	〃	2360-2480	S-SW (25°-34°)
	Mt. Kobushi	〃	2300-2460	S (28°-34°)
	Mt. Tokusa	〃	2100-2400	S-SE (26°-38°)
	Mt. Karisaka	〃	2100-2260	S-SW (25°-36°)

From the results of Table 1 and Figs. 2 and 4, we are able to conclude that the dead trees strips occur in general under the following common topographical conditions: 1) on the gentle south (SE-S-SW) slopes, and 2) in the heights of 2100–2500 m. above sea level.

In connection with the problem of the slope exposure, a report by Yatoh[2] on the

occurrence of dead trees strips in Mt. Myôjô and Mt. Chôsen (1718 m.) in Nara Pref., is highly interesting, because he reported that dead trees strips of *Abies Veitchii* and *A. Mariesii* occurred also on the south-west slope of the mountains. But the reason why the dead trees strips in these mountains appear at such low altitude is not known yet.

In relation to the reason why their occurrence is confined to the south slopes, wind and light seem to be very important as the climatic factors concerned. According to the meteorological data of Kitayama (about 10 km. SW of Mt. Shimagare), south or south-west wind prevails throughout the year, with the results that the tall trees just upwards the dead trees strips on the south slopes are exposed to strong winds. So in this case, close correlation can be expected among the direction of prevailing wind, the direction which the slope faces, and the occurrence of the dead trees strips. As to the wind condition in the Chichibu Mountains we have no data at the present time.

The effect of insolation may be also of importance for the occurrence of the dead trees strips. On the south side of a mountain, the direct rays from the sun will be able to pass through dead trees on to the area under the canopy of living trees upwards the dead trees strips, causing a rapid regeneration of young growth of *Abies* trees. For the formation of the characteristic structure of the "Shimagare" type of forest, therefore, this factor is quite possible to play an important role.

As to the altitude of the occurrence of the dead trees strips, we will only point out here that it is within the upper part of the distribution range of *Abies Veitchii* (1636-2337 m.) and *A. Mariesii* (1965-2550 m.) in the Japan Alps[3].

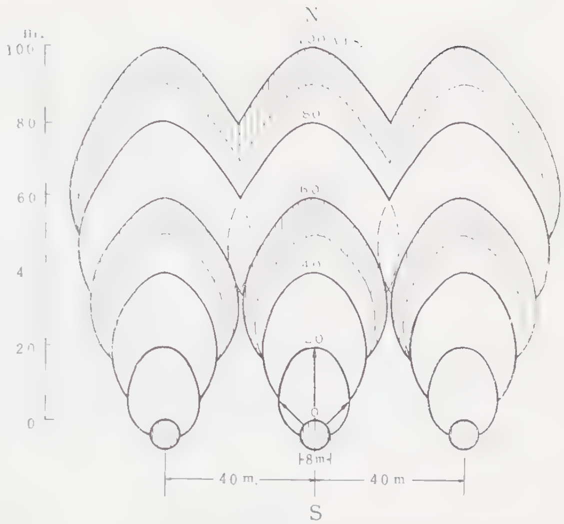
It may be said as a conclusion that wind and light will work together to make such conspicuous phenomena, but further study will be necessary to give a satisfactory elucidation of the origin of these dead trees strips.

Hypothetical process of development of the dead trees strips

As shown in the previous sections, the dead trees strips can be said as the natural phenomena which occur in a wide scale in the subalpine coniferous forest of *Abies Veitchii* and *A. Mariesii*. Now, we are here confronted with the problem how the long whitish stripes of 300-800 m. as in Mt. Shimagare were formed, and what relationship is there between such stripes and the crescent shape of dead trees strips. In order to give some solution to these problems, the authors have drawn a hypothetical diagram of development process of the dead trees strips (Fig. 5).

As the starting point of the development, a number of small circles, 8 m. in diameter, of dead trees were taken on the south slope, because the occurrence of such circles can be often observed in the forest of Mt. Shimagare, in the case when six or seven *Abies* trees died in groups. Yoshida and Yamanouchi[4] have suggested that the dead trees strip shifts upwards on the south-west slope of Mt. Shimagare at a velocity of about 1.6 m. per year. In the present case, the velocity at which dead

trees zone on the south slope moves was assumed to be 1 m. toward N, 0.75 m. toward NNE and NNW, 0.5 m. toward NE and NW, 0.25 m. toward NEE and NWW, and 0 m. toward E, W and S. If the *Abies* trees remain standing for ten years after their death, the whitish zone formed by the dead tree's trunks may have a shape of crescent after about 60 years, which is shown in Fig. 5 by the area with thin solid lines. The most spectacular examples of such crescent strip can be seen in Mt. Karisaka. In a case that several circles of dead trees occur at intervals of ca. 40 m. at the same altitude, these crescent strips will combine with each other to form a wave-like strip within 70–80 years after their start. In reality, on the south-west slope of Mt. Tokusa, a typical wave-like strip made of two or three of the crescent strips can be seen. Provided that the slope covered with the *Abies* forest is smooth and wide enough to give a large number of the crescent strips, a long stripe of dead trees, as seen in Mt. Shimagare, may be formed from such crescents in decades.



Fig, 5. Hypothetical diagram of development process of dead trees strips from the crescent-shaped ones.

On the other hand, at the site where an original circle of dead trees occurred, there is established the young extremely overcrowded growth of *Abies Veitchii* and *A. Mariesii*, which will continue their growth to mature and overmature trees, creating again the inner condition for their own death in groups. Therefore, it is not so unnatural to suppose that new circles of dead trees will occur again at the original sites within 100–120 years after the initial occurrence of the circle, and these new circles will develop into the long dead trees strips with the lapse of time. So it is quite possible on such long and wide slope as in Mt. Shimagare that several long strips of dead trees are formed by the repetition of this process.

Summary

Distribution of the crescent shape of "dead trees strips" was surveyed in the northern part of the Yatsugatake Mountains and in the Chichibu Mountains.

The structure of the crescent shape of dead trees strip, which was investigated with the line transect method, showed a close similarity to those in Mt. Shimagare, suggesting that the former is the earlier stage of development of the latter. The occurrence of these dead trees strips was confined to altitude of 2100–2500 m. and on the south (SE-S-SW) slopes of a mountain covered with the subalpine coniferous

forest of *Abies Veitchii* and *A. Mariesii.*

Some discussions were hypothetically presented about the development process of dead trees strips from the crescent-shaped ones.

The authors should express their thanks to Prof. M. Monsi, Prof. K. Hogetsu and Mr. S. Ichimura for their valuable advice. Thanks are also due to Mr. T. Saeki for his kind help during this work.

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摘　　要

1. 前報では長野県縞枯山にみられる，シラビソ・オオシラビソの立枯れからなる縞枯現象について報告したが，この縞枯の発生初期にあると思われる半月形のものは，北八ケ岳の蓼科山，横岳，雨池山，中山，硫黄岳などに，また奥秩父の[illegible]山，甲武信岳，[illegible]山，雁坂山などにもあることがわかった。

2. 帯状トランセクトによる調査の結果，これらの半月形縞枯はよく発達した直線形の縞枯に似た内部構造をもっていることが明らかにされた。またその発生は山の南側（南東—南—南西）斜面のシラビソ・オオシラビソの優群集に限られ，発生の高度は海抜 2100–2500 m. の間であることがわかった。

3. これらの半月形縞枯が時間と共に直線形の縞枯に発達する過程について考察をおこなった。

アミジグサ科植物の生活史について
I. アミジグサ，エゾヤハズ，オキナウチワの四分胞子発生*

西林長朗**・猪野俊平**

Takeo NISHIBAYASHI** and Shumpei INOH**: On the Life History in Dictyotaceae I. Tetraspore-development in *Dictyota dichotoma* (Huds.) Lamour., *Dictyopteris divaricata* (Okam.) Okam., and *Padina japonica* Yamada*.

1959 年 3 月 2 日受付

アミジグサ科植物では従来，同形同大の胞子体と配偶体との間で規則正しい世代交番が行なわれ，かつ胞子体と配偶体とは同じ時期に成熟するとされているが，Reinke (1878)[1]，Sauvageau (1897)[2]，Williams (1898, 1904)[3],[1]，Robinson (1932)[5] らによって配偶体よりも胞子体の方が多く存在することが報告され，また Carter (1927)[6]，Funk (1927)[7] によれば配偶体は見つからないで，胞子体だけが存在するといわれている。そこで著者らは瀬戸内海の塩飽群島のアミジグサ科植物について，この事実を再調査したところ，胞子体のみが発見されて，配偶体は1個体も発見されなかった。それゆえに，この地方のものは胞子体の繰返しのみによる生活史を続けているのではないかという疑問をもった。この事実の確認のために，アミジグサ，エゾヤハズ，オキナウチワの3種の胞子体を採集し，その四分胞子の完全培養を計画した。

従来，アミジグサ科植物の四分胞子発生については，Cohn (1865)[8] の報告があり，その後 Reinke (1878, '80)[1],[9]，Thuret and Bornet (1878)[10]，Williams (1904)[1]，Peirce and Randolph (1905)[11]，Carter (1927)[3]，Robinson (1932)[5]，猪野 (1936)[2]，米田・梶崎 (1953)[1] らにより詳しく研究がつづけられている。今回の著者らの実験の結果は，四分胞子の完全培養まではいたらなかったが，前述とは別の観察の際に，四分胞子の発芽の発生体とか異常発生などが見られて，従来の報告とは若干異なる新しい知見を得たので，それをここに本研究の一部として報告する。

材料と方法

実験に用いた材料は，瀬戸内海の塩飽群島産の *Dictyota dichotoma* (Huds.) Lamour. アミジグサ，*Dictyopteris divaricata* (Okam.) Okam. エゾヤハズ，*Padina japonica* Yamada オキナウチワの 3 種であり，アミジグサは 1957 年 9 月 28 日に，エゾヤハズは 1958 年 5 月 31 日に，オキナウチワは 1957 年 8 月 9 日と 9 月 28 日，および 1958 年 7 月 19 日と 9 月 13 日の 4 回にわたって，よく成熟した個体を選んで採集した。採集後，ただちに岡山大学理学部生物学教室の実験室に持ち帰って培養を行なった。成熟した胞子のついている葉状体を濾過海水で洗った後，濾過海水を満たした大型の容器に入れ，その器底にはスライド・グラスを敷いておく。24 時間後には，放出された胞子はスライド・グラス上に附着し，発生を始めているが，このスライド・グラスを別のガラス・バットに移して培養を行なった。培養液としては濾過海水をもちい，栄養塩類は加えなかった。

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* 文部省科学研究費，課題番号 407127
岡山大学理学部生物学教室植物形態学研究業績 No. 69，玉野臨海実験所業績 No. 49
** 岡山大学理学部生物学教室 Department of Biology, Faculty of Science, Okayama University, Okayama, Japan.

観　　察

1. *Dictyota dichotoma* (Huds.) Lamour.
アミジグサ

放出された四分胞子は球形，または球形が引き伸ばされたような形で，その直径の平均値は 82.19±6.99 μ である。胞子は色素体に富んでいるが，胞子の中央部には核があって色素体は少なく，色が薄くなっている (Fig. 1, A, B, C) 胞子は放出後，間もなく沈下して地物に附着するようになる。この時，胞子は第一分割壁によって二分される。二分によって生じた上側の細胞は，頂端細胞となるものであるが，下側の細胞は突起を出しはじめる (Fig. 1, D, E)。この突起は隔膜で仕切られて仮根となるが，仮根はその後，急速に伸長し細胞分裂を行なって多細胞の糸状細胞となる (Fig. 1, H, I)。胞子細胞自体はしばらくの間，容積を増すことなく第一分割壁に平行に走る二つの

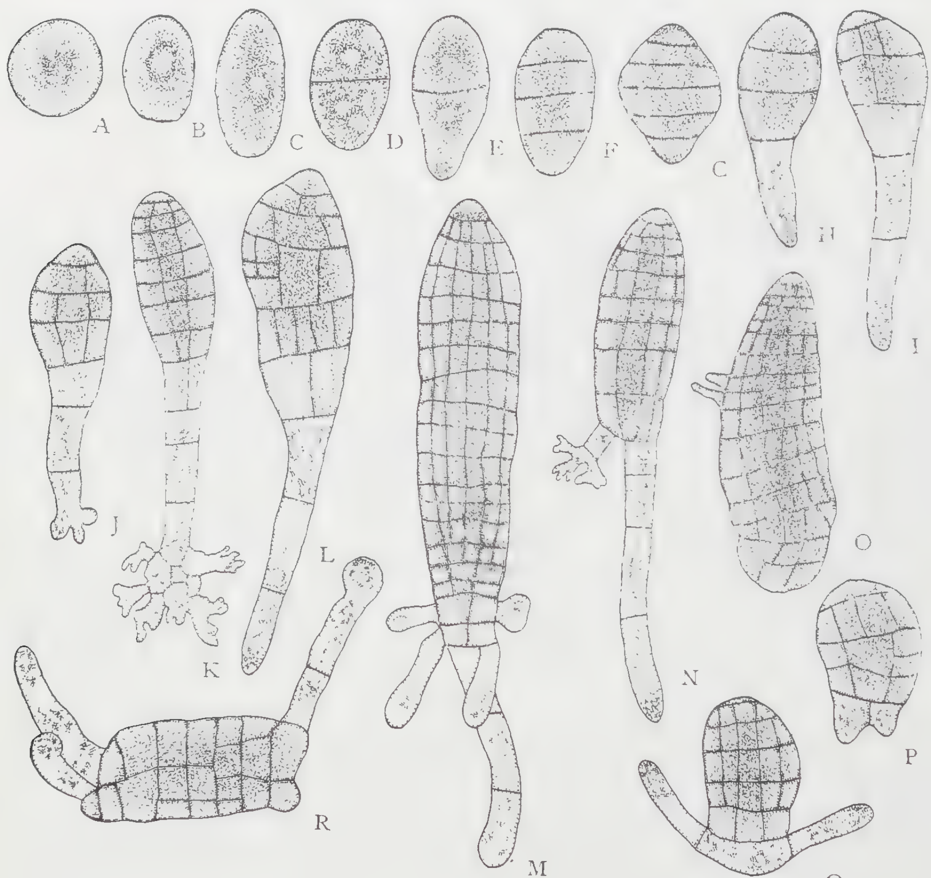

Fig. 1. Germination of tetraspores in *Dictyota dichotoma* (Huds.) Lamour. A, B, C. Liberated tetraspores with a central nucleus and chromatophores surrounding the nucleus. D, E. The first segmentation forming two cells. A rhizoid develops in Fig. E. F. The second segmentation forming four cells. G. The third segmentation forming six cells. H, I. Germlings from a culture 24 hours old. J, L. Germlings from a culture 2 days old. In Fig. J, the end of rhizoid begins to protrude. K, N. Germlings from a culture 5 days old. In Fig. K the end of rhizoid becomes sucker-shape, and in Fig. N one of two rhizoids is so. M. Germling from a culture 13 days old. Four rhizoids arise secondarily. O, P, Q, R. Abnormal germlings. (from fresh materials. ×140)

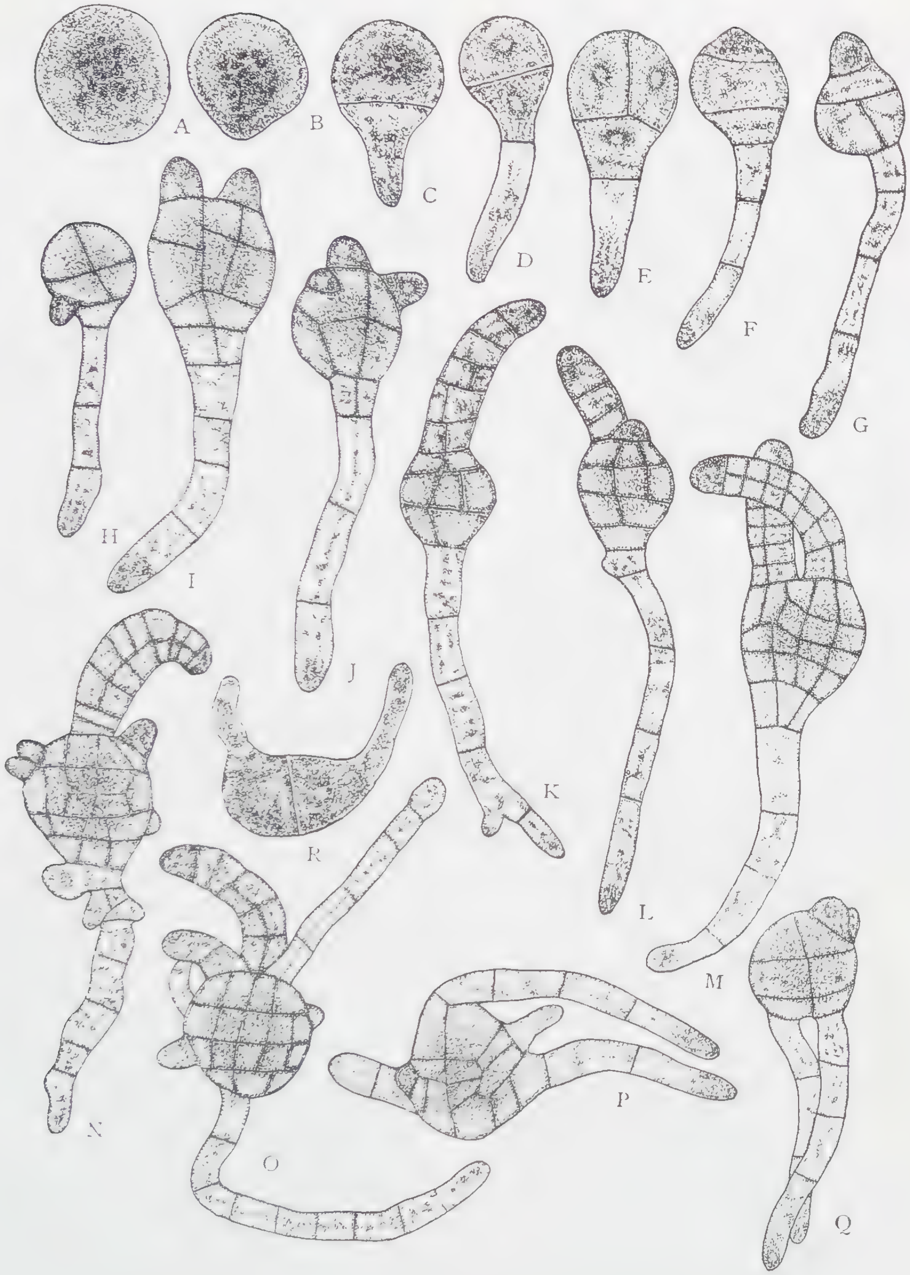

Fig. 2. Germination of tetraspores in *Dictyopteris divaricata* (Okam.) Okam.
A. Liberated tetraspore. B. The terminal protrusion developing from the body of tetraspore. C. The first segmentation forming two cells. A rhizoid develops. D, E. Germlings from a culture 24 hours old. The rhizoid develops further. F, G, H, I, J. Germlings from a culture 2 days old. In Fig. F the apex of the body begins to project and the protrusion is cut off from the body in Figs. G, H, I, J. Two protrusions are seen in Fig. I and three in Fig. J. K, L. Germlings from a culture 8 days old. M. Germling from a culture 18 days old. N, O. Germlings from a culture 11 days old. Note outgrowth of peripheral cells of the tubercle in all directions. In Fig. O the secondary rhizoids are seen. P, Q, R. Abnormal germlings. (from fresh materials. ×140)

膜により，平行に並んだ4細胞に分けられる。その後は不規則に分割壁が走り，胞子細胞は細分される。放出後2日目の発生体では，仮根の先端がふくれはじめ，その突起は大きくなって不規則な形をした吸盤状となる (Fig. 1, J, K)。このころから細胞自体も成長しはじめ，頂端細胞は細胞ら胞子分裂を盛んに行なって体はいちじるしく伸長し，円筒状となる(Fig. 1, L, M)。体が大きくなってくると，仮根のすぐ上の細胞およびさらにその上の細胞から第二次仮根が伸びだしてくる(Fig.1, M)。第一次仮根は胞子が第一分割壁によって二分された時に伸びだしてくるのが普通であるが，時にはこれが幾分か遅れて，第一分割壁に平行に走る四つの細胞壁により，胞子細胞が6細胞に分れた後にはじめて仮根が伸出してくるものもあった (Fig. 1, F, G)。また仮根を伸出することなく，胞子細胞だけが分裂，成長して相当の大きさにまで生育するものもある(Fig. 1, O)。発生体は1本の第一次仮根を持つのが正常であるが，胞子の両端から1本ずつ計2本，また同じ側で2本の仮根を出す異常なものも観察された(Fig. 1, P, Q, R)。仮根は多くの場合吸盤状であるが，糸状のものも多数観察され，また Fig. 1, N のように第一次仮根は糸状であるが，第二次仮根は吸盤状を示すものもあった。

2. *Dictyopteris divaricata* (Okam.) Okam. エゾヤハズ

放出された四分胞子は球形で，その直径の平均の大きさは 88.04±12.64μ である (Fig. 2, A)。放出後まもなく胞子は体の一端から突起を出し，その後第一分割壁が横に走り2細胞に分けられる (Fig. 2, B, C)。突起は隔膜により胞子細胞から仕切られて仮根となる (Fig. 2, D)。仮根は伸長し分裂して数細胞となる。胞子細胞自体は容積を増すことなく，不規則に分裂して多細胞となる (Fig. 2, E)。放出後2日目の発生体ではその頂端部の細胞が隆起しはじめる (Fig. 2, F)。隆起は隔膜により切り出された後，分裂をくりかえして多細胞となり葉状体を作っていく(Fig. 2, G, K)。隆起は発生体の頂端部，すなわち仮根側と反対の側に一つできるのが普通であるが，仮根と同じ側にできたり，また隆起は二つ，時によると三つ作られることもある (Fig. 2, H, I, J, L, M)。胞子の大きさが比較的大きいものに，二つまたは三つの隆起が作られるようである。Fig. 2, N, O は培養後11日目の発生体を示すが，この頃になると多くの発生体は最初の大きくなった突起のほかに，細分された胞子細胞の周辺の細胞から不規則に突起をだし，胞子細胞から沢山の角(つの)がでたような形になる。以後，発生は進まないで，ほとんどのものが死滅した。エゾヤハズの発生体においても，アミジグサの場合と同様に，胞子の両端から1本づつ計2本の第一次仮根をだすものや，一方の側から2本の仮根をだすといった異常なものが観察された(Fig. 2, P, Q, R)。培養後10日目位の発生体では第一次仮根以外に，第二次仮根が胞子細胞のいろいろな部分から不規則に伸出してくる (Fig. 2, O, P)。

3. *Padina japonica* Yamada オキナウチワ

スライド・グラス上に放出されたオキナウチワの胞子の大きさは，約 47～119μ と非常に変異に富んでいる。Fig. 3 はこの胞子の大きさを度数分

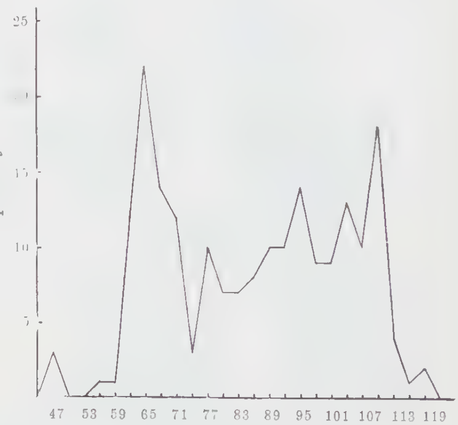

Fig. 3. Variation of liberated spores in *Padina japonica* Yamada.

布で表わしたものである。この図は二頂曲線を示し，放出された胞子は 77μ 位を境として，それより小さいものと大きいものの2群に分けられる。そして 77～119μ という胞子の大きさは，ちょうど葉状体の上にある成熟に近い四分胞子母細胞の大きさに一致しているので，小さい方の胞子が四分胞子であり，大きいものは四分胞子母細胞が四分胞子にまで成熟することなく，未成熟の状態で葉状体から放出されたものと思われる。四分胞子と母体から離れた放出四分胞子母細胞との発生の様子にはいくらかの差異が認められた。

（イ） 四分胞子の発生

放出された四分胞子は球形で，中央に一つの核を有し核の周辺部には色素体が集まっている (Fig. 4, A)。[illegible]スライド・グラスに附着し発生をはじめる。胞子は体の一端から[illegible]るとともに，[illegible]壁により2細胞に分けられる (Fig. 4, B, C)。突起は[illegible]により[illegible]たもの，[illegible]からなる糸状の仮根となる (Fig. 4, D)。仮根の伸出方向は光によって決定される。胞子細胞はその後，容積を[illegible]ことなく[illegible]て，4〜16[illegible]れる (Fig. 4, E)。放出後4日目ぐ[illegible]と，発生体の頂部の細胞の一つが隆起しはじめる (Fig. 4, F)。この突起は切りだされたが，[illegible]となり分裂，成長して新しい葉状体を作っていくが，一ケ月ぐらい培養した頃にはほとんどのものが死滅した (Fig. 4, G, H, I)。

（ロ） 放出四分胞子母細胞の発生

[illegible]のままで[illegible]母細胞は球形で，中央に一つの核を持っている (Fig. 5, A)。Fig. 5, B は中央がくびれた異常な[illegible]に発芽しないで，四分胞子嚢の中で四分胞子が形成される場合と同じように，2回のひきつづいた[illegible]を行なって，胞子内には四つの遊離核が見[illegible] (Fig. 5, C, D)[illegible]突起を出す (Fig. 5, E, F, G)。このような状態に[illegible]時間の中に胞子細胞は8〜16の小細胞に分けられる。[illegible]となる (Fig. 5, H, I, J, K, L)。放出四分胞子母細胞から生ずる第[illegible]1[illegible]で，2本または3本持つものが多数観察された。2本の仮根を持つものでは，その仮根が体の両端から出ることもあれば，[illegible]2[illegible]ている場合もある (Fig. 5, J, N, Q)。3本の仮根をもつものでは，それら[illegible]それぞれ[illegible]に向ってでているものもあれば，体の両端から一方の極では1本，他の極では2本の仮根を伸出しているものもある (Fig. 5, O, P)。Fig. 5, U では5本の仮根が見られるが，これは異常なもので，一つの胞子細胞から4本以上の仮根を出すことはひじょうに稀である。放出後3日目の発生体では胞子細胞が細分されてできた小細胞の一つが突起を出し，突起は隔膜によって仕切られた後，横裂を

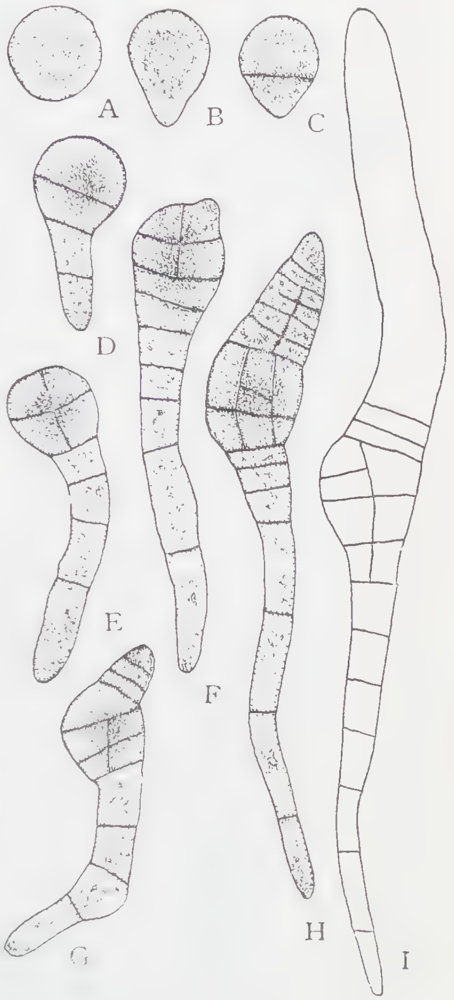

Fig. 4. Germination of tetraspores in *Padina japonica* Yamada.

A. Liberated tetraspore. B. The terminal protrusion developing from the body of tetraspore. C. The first segmentation forming two cells. D, E. Germlings with a rhizoid growing from the basal cell. F. Germling from a culture 4 days old. The apex of the body begins to project. G, H. Germlings from a culture 6 days old. The multicellular thalloid expansions and the septated filamentous rhizoids have arisen at opposite poles of the tuber-like structure. I. Germling from a culture 18 days old. (from fresh materials. ×170)

くりかえして細長くなり，後には縦裂も加わって大きな円筒状の葉状体を作っていく（Fig. 5, M, Q, R, S)。放出四分胞子母細胞から生じた円筒状の葉状体は，四分胞子から生じたものにくらべていちじるしく大きく，強健であり途中で死滅するものは少ない．培養後6ヶ月経ったものでも，なお葉状体は円筒形のままであり扇状には発達しない。円筒状の葉状体は，仮根の数には関係なく，

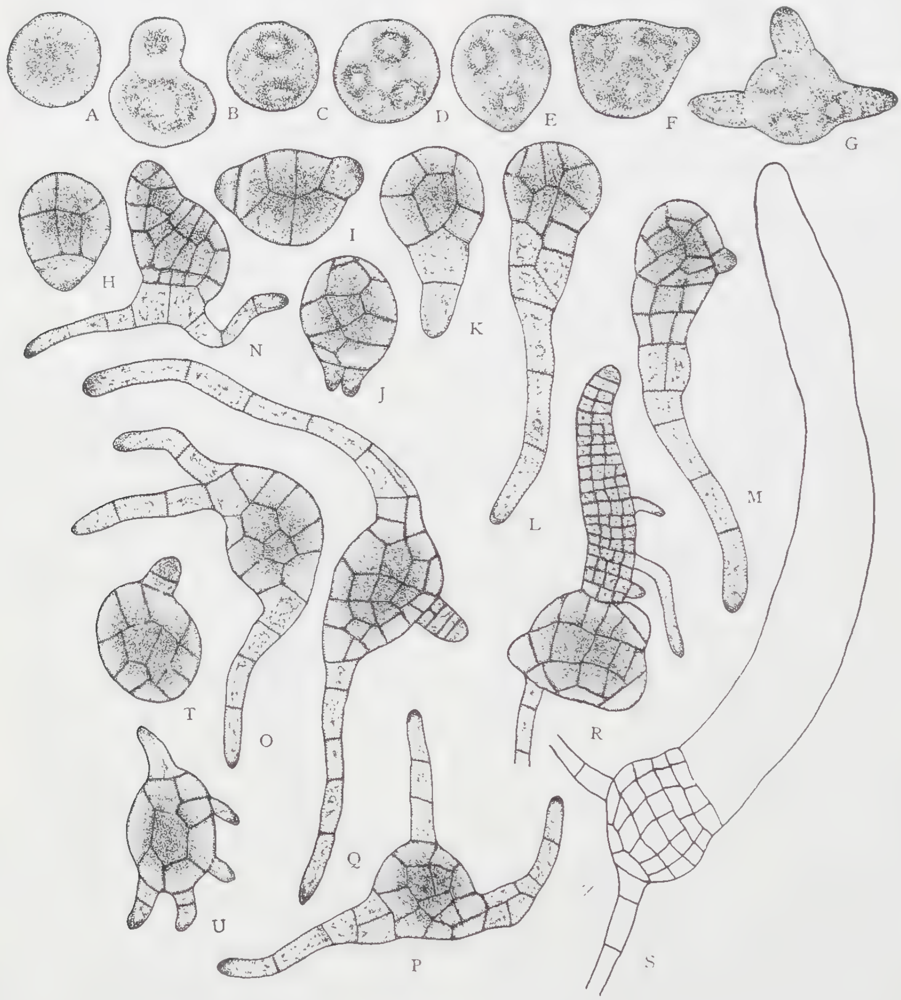

Fig. 5. Germination of tetraspore mother-cells in *Padina japonica* Yamada.
A. Liberated tetraspore mother-cell with a central nucleus and chromatophores surrounding the nucleus. B. Abnormal spore. C. Liberated tetraspore mother-cell with two nuclei. D. Liberated tetraspore mother-cell with four nuclei. E, F, G. The protrusion developing from the body of liberated tetraspore mother-cell. H, I, J. Liberated tetraspore mother-cells segmented simultaneously to produce a tubercle of 8∼16 cells. K, L. Germinating tetraspore mother-cells from a culture 2 days old. M. Germinating tetraspore mother-cell from a culture 3 days old. One of the peripheral cells of tubercle protrudes to give rise to a thallus. N, Q. Germlings from a culture 4 days old. Two rhizoids are developing at one pole or opposite poles. O, P. Germlings from a culture 2 days old. Three rhizoids are developing. R. Germling from a culture 14 days old. A well-developed thallus is derived from a tetraspore mother-cell. S. Germling from a culture 18 days old. T, U. Abnormal germlings. (from fresh materials. ×110)

一つの放出四分胞子母細胞から一つ作られるのが普通であるが，稀に二つ作られることもある。また Fig. 5, T のように仮根を生ずることなく，葉状体を作っていくといったものも観察された。

考　　察

以上の観察結果からアミジグサ，エゾヤハズ，オキナウチワの3種の四分胞子の発生様式は，それぞれの種により特徴があることがわかる。アミジグサでは第一次隔壁によって二分された四分胞子の上位細胞が頂端細胞となり，分裂をつづけて新しい葉状体をつくっていく。エゾヤハズ，オキナウチワでは放出された四分胞子は，容積を増すことなく細分されて球形の多細胞塊（cellular body）をつくり，この細胞塊の表面の一部から突起を出し，これが隔壁によって仕切られて頂端細胞となり新植物をつくっていく。この突起の数はオキナウチワではおおむね一つであるが，エゾヤハズでは一つのこともあれば，二つまた時には三つのこともあり，発生が特に進んだものには，その周辺の細胞から不規則に多数の突起を出すようになる。このようにエゾヤハズとオキナウチワの頂端細胞は，アミジグサのものよりもいくぶんか遅れて分化してくる。葉状体の生長点細胞が放射状にならび，いわゆる縁辺生長をしているエゾヤハズやオキナウチワも，その初期発生では塊状生長を行なってアミジグサと異なる生長の様式を示している。

オキナウチワでは四分胞子のほかに，四分胞子母細胞が四分胞子に成熟するまでに放出されることがある。このような四分胞子母細胞の放出と発生に関しては，Reinke (1878)[1] が *Dictyota* および *Padina* で，Williams (1904)[4] が *Dictyota* で，Robinson (1932)[5] が *Taonia* で報告している。*Padina* では四分胞子と放出四分胞子母細胞との間で，その発生には差異がないとされているが，著者らのオキナウチワについての観察では，若干の差異が認められた。オキナウチワの四分胞子は放出後まもなく突起を出し，その後，隔膜によって上下の2細胞に分けられる。放出された四分胞子母細胞は，四分胞子形成の場合と同じく，2回の核分裂の結果四つの遊離核がつくられ，その後，突起を出しはじめると同時に，いっせいに隔膜形成が行なわれて8〜16の細胞からなる多細胞塊がつくられる。そして四分胞子からはかならず1本の仮根を伸出するが，放出四分胞子母細胞から伸出する仮根の数は1本と限らないで，1, 2, 3本と変化に富んでいて，その伸出方向もいろいろである。このような事実は，放出四分胞子母細胞は四つの胞子に分離しないけれども，発生初期に四分胞子形成と同じような経過をとって4核となるので，四つの胞子の集合体と考えるとよく理解される。フークス目のホンダワラ属植物の卵は8核が卵細胞中に散在している状態で放出され，受精後，不用の7核は退化して1核だけが残り，2回の核分裂の後に卵の下端に小さいレンズ状の仮根細胞が切り出され，この細胞から仮根を伸出する。そして一つの造卵器の中に，ヒバマタのように八つの卵が形成されないで，8核を含んだ1卵がつくられるだけなので，8卵の集まったものと見なされる。しかし，その仮根は卵の下端の仮根細胞からつくられ，集まった8卵のおのおのから自由につくられないので，卵全体が一つのSyncytiumとして統一され，一極に仮根伸出の極である仮根細胞が決定される。すなわちホンダワラ属植物の卵の場合は，8核があっても1卵としての完全な統一体と考えられる。これに対してオキナウチワの放出四分胞子母細胞も四つの胞子の集まったものと見なされるが，ホンダワラ属植物の卵よりも，統一性が不完全で，なお四つの胞子のおのおのが自由に発生を始めようとする。そのため仮根伸出の極が一つに定まらないで，しかも仮根は1本と限らず，2本または3本も伸出するものがあり，その伸出方向も不規則になる。しかし，新植物をつくってゆく頂端細胞の突起は仮根の数に関係なく一つつくられるだけなので，この頃になると放出四分胞子母細胞内の1胞子は完全な一つの発生体としてはたらいている。

Robinson (1932)[5] は *Taonia* の四分胞子も，放出された四分胞子母細胞もともに，仮根の伸出方向は光によって決定され，放出四分胞子母細胞から2本以上の仮根が出る場合，それらは一つの極に集まって出ると述べているが，オキナウチワでは四分胞子の仮根の伸出方向は光によって決定されるけれども，放出四分胞子母細胞の場合は光に関係なく，2本以上の仮根を出すものでは，それらの仮根は不規則に伸出して，一つの極に集まって出るということは少ない。放出四分胞子母細胞

は四つの胞子の集合体なので，四つの胞子に相当する部分にそれぞれ光が影響を与え，それらの部分が自由に仮根を伸出して発生を進めていこうとする。それゆえ，放出四分胞子母細胞では，光が仮根伸出に対する決定的な要素とならないのである。

アミジグサでは吸盤状の仮根と糸状の仮根とが観察されたが，同一個体でも第一次仮根が糸状であるのに，第二次仮根が吸盤状になっているのが見られた。このことからアミジグサの仮根は，緑藻類で広く知られているように，環境条件によって吸盤状になったり，糸状になったりするもので，糸状の仮根は不良環境のために発生した異常型と思われる。

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Summary

The germination of the tetraspore in *Dictyota dichotoma* (Huds.) Lamour., *Dictyopteris divaricata* (Okam.) Okam. and *Padina japonica* Yamada has been observed. It shows certain differences among three species. In *Dictyota dichotoma* the upper segment which is formed by the first division of the spore becomes the apical cell of the thallus. In *Dictyopteris divaricata* and *Padina japonica* the germinating spores first produce a multicellular oval body. Thereafter the oval body produces a projecting cell, which becomes the apical cell of the new plantlet. In *Padina japonica* a projecting cell is made, but in *Dictyopteris divaricata* 1～3 protrusions are produced at first and many later.

The occasional liberation and germination of the entire sporangial contents were observed in *Padina japonica*. There are some differences in germination between tetraspores and liberated tetraspore mother-cells. Those differences are attributed to the fact that the liberated tetraspore mother-cell is the aggregation of four tetraspores.

短　報

植生地図国際シンポジウムについての短報

宮　脇　　昭*

Akira MIYAWAKI*: Ein kurzer Bericht über das Internationale Symposion für Vegetationskartierung vom 23–26. März 1959 in Stolzenau/Weser, Deutschland

1959 年 4 月 20 日 受付

国際植物地理・生態学会 Internationale Vereinigung für Vegetationskunde—Association intarnationale de phytosociologie—International society for plant geography and ecology. Präsident Dr. W. C. De Leeuw in Leiden, Holland, Sekretär Prof. Dr. R. Tüxen in Stolzenau, Deutschland の主催による植生地図についての Internationales Symposion が，3 月 23 日～26 日にわたって当地で開催された。19 か国百数十名の専門研究者により焦点を植生地図にしぼり，各国の今までの研究成果と今後の課題について熱心に討議された。はじめてこの国際学会の主催する Symposion に出席する好機を得た筆者は，4 日間 40 講演（別に夜は特別講演 4）とのべ 12 時間以上の討論が行なわれたこの会から深い感銘と刺激とを与えられた。しかもこの分野の研究が，ヨーロッパ各国ではすでに長い研究の歴史と数々のすばらしい成果により，今や特定の人たちや国々の独占物でなく，広く地球上の各国に深い根を張って，発展を続けている現状を再認識させられた。

以下簡単にこの Symposion について報告したい。この小文を通じて比較的にこの分野の研究に後進的なわが国に，とくにヨーロッパ大陸を中心とした各国の植物社会学研究情況の一端が報告できれば幸である。

講演および討論は 1. Grundlagen und Methoden der Vegetationskartierung, 2. Wissenschaftliche Ergebnisse der Vegetationskartierung, 3. Wirtschaftliche Anwendungen der Vegetationskartierung の 3 分科に分けられ，4 日間 8.00—19.00 時のあいだ昼食時の休憩を除いて，休みなく行なわれた，座長 Prof. Dr. Welten, Bern (Schweiz), Prof. Dr. Küchler, Kansas (U.S.A.), Prof. Dr. Horvat, Zagreb (Jugoslawien), Prof. Dr. Schmithüsen, Karlsruhe (Deutschland), Prof. Dr. Emberger, Montpellier (Frankreich), Prof. Dr. Matuszkiewicz, Warschau (Polen) により会は進行された。

国際学会事務局長 Prof. Dr. R. Tüxen の Symposion の意義と各国からの参加者に対する謝辞を主とした開会の辞についで，Schwickerath, Aachen の講演を皮切りに会が開かれた。

第 1 分科では各国の今迄の研究成果を例に植生地図の原理と方法論について，1 日半にわたりもっとも活発に論議された。すなわち Küchler (U.S.A.) Scamoni (D.D.R.), Noirfarise (Belgien), Horvat (Jugoslawien), Gaussen (Afrika) および Molinier は地中海の海藻植生について発表し，討論がおこなわれた。さらに所長 R. Tüxen 以下 Trautmann, Lohmeyer,

* 横浜国立大学生物学教室　Biologisches Institut, Staatliche Universität zu Yokohama, Kamakura. z. Zt. Bundesanstalt für Vegetationskartierung, Stolzenau/Weser Deutschland.

Walther, Wenzel らドイツ国立植生地図研究所員による野外における調査，研究の原理と方法，航空写真の植生地図への利用法とその限界，植物社会学研究志望者への植生地図の画き方についての指導法，植生地図作製上の技術上の問題点，さらに種々の植生地図の Typen とその調査および各 Typen の科学的意義などについての発表。さらに広く植生地図全般について意見がかわされた。

植生地図は何を基礎にして画かれるべきかという昔からの根本問題が，ふたたび一応問題になり，討論された。すでに古くは v. Humboldt 1805-16 および Drude 1885-1928, Brockmann-Jerosch 1907-34, Rübel 1912-39 らにより画かれた Physiognomie による方法，これは植物を知らなくても画けるので，自然地理学者に重宝がられて来た。優占種による方法，これも画きやすいが，はたして優占種はつねにその群落を，またその生育地の環境条件をただしく反映しているだろうか，という植物群落上の根本問題が，ここで討論された。さらにこの問題に関聯して Standortskartierung と Vegetationskartierung との関係についても会場内に展示してある各国の地図を例に討論された。

結局植物社会が，種々の共存により成立っている以上，植物社会の具体的また抽象的な存在を表わす植生地図は，当然種の組み合せ (Kenn-Arten, Trenn-Arten)—Assoziation またはその下位もしくは上位単位—を基礎に画くのがもっとも妥当であろうという結論が再確認された*

なおこのことについては，さらに次の Symposion までに各自多面的に植生地図を研究し，その成果を検討しようということになった

第2分科の植生地図の科学的成果については Apinis (England), Major (U.S.A.), A. O. Horvàt (Ungarn), Majovský, Jurko (Tschechoslowakei), Bertović (Jugoslawien), Doing Kraft (Holland) らによる各地の植生と種々の梯尺による植生地図の研究成果が発表，討論された。Zonneveld (Holland) は，特に同国の河岸地帯の土性図と植生地図との比較研究成果について興味ある発表を行なった。さらに J. Tüxen による植生地図を手がかりとしての Altlandschaft の研究例および Schmithüsen による種々の梯尺による植生地図の植物社会学および隣接科学への寄与について，同氏の南アメリカの研究成果からの理論的裏づけなどは注目をひいた。

日本の植生についても強い関心が持たれた たとえば鈴木の月山の雪田植生（筆者代読）についても Emberger, Gaussen, Küchler, Barkman (Holland) らから，日本の森林植生の特異性などについて質問があった

近年南アメリカ，アフリカには相当数のヨーロッパの学者が進出し，植物群落の研究が進んでいるにもかかわらず，東南アジアは，比較的遅れている。したがって日本の研究者による同じ基礎にたった研究成果が，強く期待されていることが，Symposion 前後の公私の質疑を通して感じられた

第3分科は植生地図による研究成果の林業，牧野，農耕地および塩害草原の土地改良， Wasserwirtschaft（発電，運河，灌漑，排水），風害防止，自然保護地域など産業の各分野への応用実例と，その基礎研究について，Frau A. Matuszkiewicz (Polen), Long (Frankreich), Fukarek, Wraber, Ziani (Jugoslawien), Mráz, Samek (Tschechoslowakei), Wagner (Österreich) de Boer, Tideman (Holland), Marschall (Schweiz) Meisel, Seibert, Frau Steubing usw. (Deutschland) により講演，討議された。

Bundes ministerium für Verkehr の Barz はとくに発言を求め，ドイツ国立植生地図研究所の研究成果が，今までドイツ産業界に貢献した実例をあげ，この分野の今後の発展を期待すると述べた。

Horvat の提案で，この機会にヨーロッパ大陸の植生地図作製に着手することにきまり， R. Tüxen を中心に Braun-Blanquet, Emberger, Horvat, Noirfalise Pawlowski で同地図作製委員会が組織された。

第1夜は，ドイツ農林省* 主催の晩さん会が開かれ，農林大臣代理の Dr. Offner はじめ各界代

* この問題は，ヨーロッパ大陸ではすでに少なくとも 10 年前から意見がほとんど一致している。

* ドイツは戦後，教育の地方分権が行なわれ，日本のように中央に統一的な文部省は存在しない。

表の[illegible]のち，おそくまでなごやかな[illegible]ついた

第 2 夜は Bundesanstalt für Vegetationskartierung とその土壌断面博物館の見学。第3, 4 夜は，アフリカ，オーストラリア大陸をはじめ，インド，タイ，南アメリカ大陸の植生および地中海の[illegible] 70 m.[illegible]，[illegible]る特別講演が Emberger, Gaussen, Küchler, Molinier により行なわれた。

講演および質疑応答は，大部分ドイツ語で，ついでフランス語で行なわれたことも，この分野の科学[illegible]ヨーロ[illegible]，ヨーロ[illegible]で発展して現在に至った歴史的な姿がうかがえて興味深い。

この Symposion は，国際会議の通念としては，[illegible]それだけに植生地図という日本の植物学者には比較的疎遠があった問題に焦点がしぼられ，この分野の研究者によりつっこんだ討論が行なわれた。したがって実質的には大きな成果をあげ，今後の研究の推進に新しい力を吹きこんだことであろう。

全討論を通じて感じられたことは，できるだけ速かに，世界の研究者が同じ研究基盤に立脚して，[illegible]された[illegible]法で地球の植生地図を作りたいということである。

植生地図は，群落調査の片手間に簡単に画かれるような安易なものではなく，十分な群落調査の[illegible]，その基盤にたって，客観的に慎重に画かれなければならない。そしてこのようにして得られた成果こそ，科学の発達と応用分野に貢献するものであることを，深く認識させられた。

なお，この Symposion の講演および討論は，"Bericht über das Internationale Symposion für Vegetationskartierung" im Verlag Engelmann, Weinheim に掲載される。

Zusammenfassung.

Das Internationale Symposion für Vegetationskartierung wurde von der Internationalen Vereinigung für Vegetationskunde vom 23. bis 26. März 1959 in Stolzenau/W., Deutschland, als bisher größter pflanzensoziologischer Kongreß durchgeführt. Es ist daher angebracht, einen kurzen Bericht zu geben über einige Ergebnisse dieser Zusammenkunft der bedeutendsten Fachgelehrten aus dem Bereich der Vegetationskartierung, die aus vier Erdteilen nach Stolzenau gekommen waren. Mehr als 40 Fachvorträge und eingehende Diskussionen, die während des Kongresses gehalten wurden, brachten einen so gut wie erschöpfenden Überblick über den Stand der Vegetationskartierung, ihre Grundlagen und Methoden, ihre wissenschaftlichen Ergebnisse und ihre Anwendung in der Praxis der meisten Länder Europas und auch in Übersee.

Wir hoffen, daß dieser kurze Bericht für unser Land, das in diesem Teilgebiet der Botanik weniger fortgeschritten ist als Europa, Anregungen zu geben und zum Fortschritt auf diesem Gebiet der Naturwissenschaft beizutragen vermag.

雑　　録

Troschin, A. S.: Das Problem der Zellpermeabilität. Aus dem Russischen übertragen von Werner Höpper. Mit 118 Abb. und 82 Tab. im Text. 396S. Gustav Fischer, Jena 1958, DM 40.20 (邦価約 4,000 円).

細胞の透過性は従来主として"膜説"の立場から考えられてきた。膜説では，原形質と外界との間に半透性の原形質膜を仮定する。そして外液と細胞との間の物質の分配はこの膜の構造と機能によって決定されると考える。しかし実験技術の進歩につれてこの膜は必ずしも半透性ではなく大部分の物質を比較的たやすく透過させうることが明らかとなってきた。そこで古典的な膜説は種々に改変される必要にせまられた。近来盛んにとなえられている carrier hypothesis (Trägertheorie) はその代表的なものであろう。しかしこれも結局は存在の有無が確認されていない Träger という仮想実体を導入することによって，物質透過の現象をますます不可解なものにしたようである。

すでに今世紀の始めに Fischer らは膜説に批判を加え，細胞による物質の吸収は原形質自体の膠質化学的な現象と考えるべきであるとした。この考え方はさらに Lepeschkin, Fischer, Nassonow らによって発展され，原形質と外液との間の物質分配の原理は原形質が外液に対して一つの"相"(Phase) の性質をもつことにもとずくもので，膜による選択的拡散でないことが主張された。しかしこれらの主張の弱点はこの相の性質について具体的な説明を与え得なかったことである。

本書は透過性に関する全般の問題を取扱っているが，とくにこの相の具体的な像を多数の実験的事実から与えている点で注目される。著者 Troschin は現在レニングラードのソヴィエト科学アカデミー動物学研究所の細胞学教授であって原著は 1955 年ロシア語で書かれ，本書はその独訳である。原形質は一種の複合コアセルベートであるという Oparin, Guilliermond, Makarow らの見解を基礎として，彼はまずコアセルベートと原形質の一般的性質の類似性からとき起し，原形質における物質分配が非電解質，電解質，無機物，有機物を問わずすべてコアセルベートの場合と同一原理にもとずいておこなわれることを示そうとする。

周知のように一つの物質は2種の相接して互いにまざらない液体の間に一定の比をもって分配されるが，同様のことが複合コアセルベートとしての原形質と外液との間にもなり立つ。ある物質の原形質への分配は原形質と外液との間の溶解性の差のほかに，原形質コロイドの吸着性と化学的な親和力によっても大きく支配される。これらの3因子を総合して著者は"Sorption"(収着)と名づけ，Lepeschkin, Nassonow らの説を"膜説"に対して収着説 (Sorptionstheorie)，あるいは原形質説 (Protoplasma-Theorie) と呼んでいる。

たとえばカエルの縫匠筋のカリウム含量は外液(リンガー液)からカリウムを除いてもなお 0.075 M/l. の濃度で筋中に保たれる。これは"化学的に結合"しているカリウム量を示すものと考える。外液のカリウム濃度を 0.01 M/l. に上げると筋のカリウム量は急に増大し，0.12M/l. となる。この際の増加量 0.45 M/l. は筋原形質コロイドによる"吸着量"である。外液のカリウム濃度をさらに上げると筋肉中のカリウム量の増加率は減り，外液の濃度増加と比例するようになる。すなわちある外液濃度以上ではカリウムは原形質と外液との間に一定の比をもって分配されることがわかる。カエルの筋肉ではカリウムの分配比が 0.45 であるがこのことは原形質に対するカリウムの"溶解性"が水に対するそれの約半分であることを示すものである。同様の現象は他のイオンでもまた神経その他の組織においても見られるが，一般に分配係数は1より小さい。このことは，溶液中と同様に"自由に動きうるイオン"の量が原形質中では外液のそれよりも少ないことを意味し，さらにまたイオンが濃度勾配にさからって吸収されるという考え方の誤りであることを指摘することにもなる。

本書は主として動物細胞によって得られた知見をとり扱い，液胞をもつ植物細胞の場合の説明はすくない。植物細胞の場合は原形質は外側で外液に接すると同時に内側では細胞液に接し，しかも細胞液のイオン濃度は外液のそれより一般にはるかに大きい。このような液胞へのイオン集積機構を"収着説"でどのように説明するかは今後の問題であろう。いずれにせよ，本書に紹介された収着説は細胞透過性に関する既成概念への再検討を迫り，さらに[illegible]もの，あるいはその中の物質の存在状態へと関心を向けさせる。そういう意味で透過性の研究者はもちろん生理学，生化学，細胞学に従事する人達も本書を読んで得るところや考えさせられる点が多いと思う。要するに本書は透過性をいままでの考え方とは異なった観点から総合的に取扱った点でまことに興味深く，またユニークな内容をもっているといえる。なお本書にはわが国に比較的疎遠であるソヴィエトの文献が多数引用されていることを付記しておく。 (神谷宣郎)

韓 国 植 物 学 会

韓国植物学会は 1958 年に発足し，4 月に会誌 The Korean Journal of Botany 第 1 巻第 1 号を出している。私の友人洪元植理事から送られた会誌によると，[illegible]その前にあり，The Korean Journal of Biology (1957 年に第 2 巻)を出し，1957 年 11 月 30 日，12 月 1 日の 2 日間高麗大学校で，第 12 回，植物学会研究発表会を行なっており，その他大学もちまわりで，[illegible]ているようで，1号の末尾には研究発表会の記録と質疑とともに，例会の記録（要旨，質問，答）がのっている。会長は[illegible]，編集委員長は[illegible]，その他副会長，理事，監事，幹事などの役員がこれを運営している。本部は Seoul 大学校文理科大学植物学教室にある。

第 1 号の内容をみると，論文が 3 つ（酵母菌の銅抵抗変異に対する DNA の影響に関する研究，食用菌類のアミノ酸について，モクゲンジの海流による伝播），資料が 2 つ(韓国産野生繊維植物について，韓山の特記すべき植物)のっている。引用文献にはわが国のものも多いが，概して不充分のようである。洪元植氏の手紙によると，文献の入手難はちょうどわが国の終戦直後のような状態らしい。 (沼 田 真)

日本植物生理学会の創立

植物生理学のめざましい進展にもかかわらず，わが国にこれまで植物生理学に関する専門学会組織の確立されることなく今日に至ったのはむしろ不思議といってよい。これまでにも毎年植物学会の大会ごとに開催地教室有志の世話で植物生理学者懇談会が開かれてはいたが，一層定常的な学会組織の必要は関係者の誰しもの痛感するところであった。

他方，先年パリで開かれた第 8 回国際植物学会(1954 年 7 月)において諸国からの出席者の間で植物生理学の国際的な連合機関 International Association of Plant Physiologists (国際植物学連合) を設立しようとする予備的協議が行なわれているが，この動きはその後も引つづいており来る 8 月にカナダで開かれる第 9 回国際植物学会の際にその実現が期待されるところまで進んでいる。その準備ならびに結成後の運営のための協議会はイギリスの T.A. Bennet-Clark 教授を会長とし，スカンジナヴィア植物生理学会代表 H. Burstrøm，インド植物生理学会代表 J. J. Chinoy 教授，カナダ植物生理学会代表, P. R. Gorham 教授，フランス植物生理学会代表 R. Ulrich 教授，アメリカ植物生理学会代表 F. W. Went 教授，さきの準備協議会の会長として K. V. Thimann 教授，幹事 B. Kok 博士により構成され着々準備をすゝめている。この国際生理学連合は世界の植物生理学者(ならびに植物生理学会)の間の連絡をはかり，植物生理学の国際的シンポジウム，または国際会議を企画するなどにより，この学問分野の進展に寄与することを目的とし，また成立の上はただちに国際生物学連合 (International Union of Biological Sciences, IUBS) に参加してこの機構を通じての諸国植物生理学者の研究上の連絡や，意見の表明，達成を助けようとする

ものである。そしてその構成は各国それぞれの植物生理学会を横に結ぶ連合的機構として構想され，すでにその主旨により，わが国にも上の協議会に代表を送り，この運動に参加協力するようにとの熱心な勧請状が送られて来ていた。(さきに本誌第 71 巻 840 号 p. 233，に報ぜられたインド植物生理学会(1958 年発会) の発足もこの線にそった一つの出来事と云うことができよう)。

このような内外の情勢に対応すべく，日本植物生理学会を結成しょうとの機運は本年はじめ頃から加速度的に高まり，遂にこの 1959 年 4 月 4 日創立のための発起人会が東京で開かれ，同日をもっていよいよ「日本植物生理学会」は誕生した。本会の創立に当っては，これまで理科関係の研究室における植物生理学の研究と，農学，農芸化学，林学，水産学，薬学，などの関連分野の研究ならびに実際とがあまりにも隔絶された関係にあった実情を是正すべく努力がはらわれ，この主旨に賛同して，上記各部門にわたって約 215 名の植物生理学者が発起人として参加されている。同会の評議員 (20 名)の構成についても同じ考慮が払われている。本会がわが国ならびに世界の植物生理学に如何ほどの貢献をするかは一に今後の実績にかかっているのであるが，それについて当事者らは全国の植物生理学者（広い意味における）の参加協力と，植物学者一般の温かい支援とを希望している。なお，学問の性質上，この会が日本植物学会，その他の関連学会と互いに密切な連けいを保ちつつ進んで行くことを念願していることは云うまでもない。

同会の第一の事業としては日本の植物生理学の成果(原論文)を世界に発表するための欧文誌（季刊）仮称 Plant and Cell Physiology を発刊すること。これについては基金の一部がすでにアジア財団から寄贈されて居り，今年内に第一号を出すことを目標に準備がすすめられている。なお，特定の研究題目について国内シムポジウムを随時開催することが考えられており今秋東京においてその第一回を行なうべく，目下準備中である。

なお，初年度の会長は京都大学理学部の芦田譲治教授，幹事長は，東京大学教養学部の八巻敏雄助教授，上記協議会への代表は東京大学応用微生物研究所の田宮博教授。学会事務所は京都市左京区北白川京都大学理学部植物学教室内。入会希望者は金費 500 円をそえて同所に申込まれ度しとのこと。　　　　（高 宮　篤）

正　　誤

先月号 (Vol. 72, No. 851) 223 頁の左欄一番下の一行は右欄の Table の上へ入ります。誤植をつつしんでお詫びします。

編集幹事よりおねがい

たびたび御注意しておりますが，まだ著者校正のさいに原稿以外に追加される方があります。これは校正の誤り，発行の遅延，学会経費の負担などの原因となっておりますので，今後このようなことのないようおねがいいたします。やむをえず追加されるときは，編集部で必要と認めたばあいに限り，追加することがあります。このばあいの必要経費は著者負担となります。

なお，投稿のさいには，投稿の注意（Vol. 72, No. 847）をよくお読みの上投稿してください。

本　会　記　事

日本学術会議第 5 期の選挙は 11 月 10 日に行なわれますが，本会から服部静夫，芦田譲治の 2 氏を候補者として推薦することを評議員会で決定しました。

Choline Sulfate Ester as Sulfur Source for the Growth of Fungi

by Michiko ITAHASHI*

Received February 26, 1959

The modes of metabolism of sulfur cmpounds in organisms are actually different from one to another. While, many plants and micro-organisms can grow up by taking only inorganic sulfate as sulfur source, animals must take S-containing amino acids for growth and maintenance. Inorganic sulfate taken up by micro-organisms and higher plants is changed into sulfate esters or other organic S-containing compounds. In the course of the reaction forming S-containing amino acids from inorganic sulfate, reduction of sulfate and combination of an inorganic sulfur compound with an organic compound should take place successively, although the whole reaction aspect has not yet been elucidated.

Choline sulfate ester $(CH_3)_3N^+CH_2CH_2OSO_3^-$ which was inferred by Raistrick and Vincent[1] to be an intermediate substance of sulfur metabolism, has been isolated by Woolley and Peterson[2] from *Aspergillus sydowi.*

In the previous papers[3][4][5] it has been reported by F. Egami and the present author that choline sulfate ester is effective for the growth of *Aspergillus oryzae* as a source of sulfur. They have also recognized the production of choline sulfate ester from inorganic sulfate in the mycelium of *Aspergillus oryzae* by using S^{35} as a tracer element. J. de Flines[6] also identified choline sulfate ester in the mycelial extract from *Penicillium chrysogenum.*

The present paper deals with the utilization of choline sulfate ester as source of sulfur in several kinds of fungi.

Experiments and Results

The synthetic media used are as follows:

Solutions

Basal Medium-1 (BM No. 1): One liter containing; 50 g. of sucrose, 10 g. of NH_4NO_3, 0.2 g. of KH_2PO_4 and 0.01 g. of $FeCl_3$ (pH 5.5).

Basal Medium-2 (BM No. 2): One liter containing; 80 g. of sucrose, 2 g. of

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$(NH_4)_2HPO_4$, 2 g. of KH_2PO_4, 0.2 g. of $CaCl_2$ and 0.01 g. of $FeCl_3$(pH 6.8).

Basal Medium-3 (*BM No. 3*): One liter containing; 40 g. of glucose, 4 g. of K_2HPO_4, 4 g. of asparagin, 2 g. of $(NH_4)_2HPO_4$, 0.2 g. of $CaCl_2$ and 0.01 g. of $FeCl_3$ (pH 6.6).

Choline Sulfate Ester Solution: One liter contains 18.3 g. of choline sulfate ester (M/10) and 20.3g. of $MgCl_2 \cdot 6H_2O$ (M/10).

Magnesium Sulfate Solution: One liter contains 24.6 g. of $MgSO_4 \cdot 7H_2O$ (M/10).

The solutions are sterilized by steaming for 30 min. on three succesive days and stored at room temperature until use.

Culture Medium

Each medium was prepared by mixing the above-mentioned stock-solutions in the following proportions:

Medium-1 (*M No.1*):

M. No. 1-CS

- BM No. 1 .... 90 ml.
- Choline sulfate ester solution .... 10 ml. (M/100)

M. No. 1-SO_4''

- BM No. 1 .... 90 ml.
- Magnesium sulfate solution .... 10 ml. (M/100)

Medium-2 (*M. No. 2*):

M. No. 2-CS

- BM No. 2 .... 95 ml.
- Choline sulfate ester solution .... 5 ml. (M/200)

M. No. 2/SO_4''

- BM No. 2 .... 95 ml.
- Magnesium sulfate solution .... 5 ml. (M/200)

Medium-3 (*M. No.3*):

M. No. 3-CS

- BM No. 3 .... 95 ml.
- Choline sulfate ester solution .... 5 ml. (M/200)

M. No. 3-SO_4''

- BM No. 3 .... 95 ml.
- Magnesium sulfate solution .... 5 ml. (M/200)

The medium (50 ml.) was accurately measured into a series of 300 ml. conical flasks plugged with cotton, and sterilized by heating in flowing steam for 30 min. on three successive days. The flasks were then inoculated with a pure culture of the fungus to be tested, and incubated at 28° for a requisite period of days. The mycelium from each species was separated quantitatively by filtration on a

Buchner funnel, and was thoroughly washed with warm water. The mycelium was then dried to constant weight *in vacuo* and weighed. The filtrate and the washings were made up to a total volume of 50 ml., and analyzed by the methods described below.

Ten ml. of the culture-filtrate were accurately measured into a beaker, and acidified with 2 ml. of HCl (6 N); and 1 ml. of $BaCl_2$ (10%) and 10 ml. of H_2O were successively added. The vessel covered by a watch glass to lessen evaporation, was gently boiled for 20 min. In the course of this treatement, choline sulfate ester was quantitatively hydrolyzed to produce a precipitate of $BaSO_4$, which was weighed as usual.

Table I shows the results obtained with six species of moulds belonging to Aspergillales.

Each flask containing 50 ml. of M. No. 1-CS (or M. No. 1-SO_4'') with a sulfate content equivalent to 16 mg. sulfur/flask, was inoculated with a culture of the mould species to be tested. Figures in Columns 5 and 9, indicate, in weight of $BaSO_4$, the quantities of organic and inorganic sulfate utilized by the mould during the incubation period (i. e., the difference between the amount of sulfate originally present in the medium and that remaining in the residual culture medium).

The moulds used in this set of experiments included three species of *Aspergillus*, one species of *Monascus* and two species of *Penicillium*. The experimental results show that all the froms belonging to these genera readily utilize choline sulfate ester as sulfur source, giving a better growth as compared with that obtained with inorganic sulfate as sulfar source; the only exception being the case of *Penicillium notatum*. From the fact that in these moulds, inorganic sulfate ion was not detected in the residual culture medium when choline sulfate ester was used as sulfur source, it will be concluded that choline sulfate ester must have been absorbed as

Table I

Organism	Catalogue No.*	Weight of dry mycelium (mg.)	Incubation period (days)	Utilization of choline sulfate		Weight of dry mycelium (mg.)	Incubation period (days)	Utilization of inorganic sulfate	
				Sulfate utilized ($BaSO_4$ mg./flask)	%			Sulfate utilized ($BaSO_4$ mg./flask)	%
Aspergillus niger	4407	375	9	12	10.3	370	9	5	4.3
Asp. sydowi	4402	895	9	100	86.0	795	9	45	38.8
*Asp. oryzae***		670	6	37	31.9	660	6	25	21.5
Penicillium notatum	4640	510	11	20	17.2	650	11	30	25.8
P. chrysogenum	4626	690	11	25	21.5	550	11	36	31.0
Monascus purpureus	4513	632	14	12	10.3	550	14	9	7.7

* Obtained from the Institute for Fermentation, Osaka. (Table I—IV)

** Obtained from the Faculty of Science, Nagoya University.

Initial amount of choline sulfate (or inorganic sulfate) in each flask; 116.5 mg. (on $BaSO_4$ basis)

such, i. e., without previous hydrolysis.

Table II shows the results obtained with two species of *Neurospora* belonging to Pyrenomycetes and several other species belonging to Fungi Imperfecti. Each flask containing 50 ml. of M. No. 2–CS (or M. No. 2–SO_4'') with a sulfate content equivalent to 8 mg. sulfur/flask, was inoculated. This group also readily utilized choline sulfate ester as a sulfur source and showed again a growth similar to that observed with inorganic sulfate as sulfur source. For every mould examined, except for *Alternaria tenuis*, no inorganic sulfate was detected in the residual medium which had initially contained choline sulfate ester as sulfur source. In the case of *Alternaria tenuis*, 39.2 % of choline sulfate ester and 26.8 % inorganic sulfate ion found in the residual medium. This probably means that 34 % of choline sulfate ester was absorbed and 26.8 % of the remaining (66 %) was hydrolyzed by the mould.

Since it seemed very likely that *Alternaria tenuis* would utilize as sulfur source the sulfate ions liberated through hydrolysis of choline sulfate ester, another experiment was made to check this point. A number of conical flasks (500 ml.) containing 50 ml. of M. No. 2–CS were inoculated with *Alternaria tenuis* and, after incubating at 28° on a shaker for five days, the sulfate remaining in the medium was determined. In this case, contrary to expectation, no inorganic sulfate ion was found in the residual medium, although this was a consumption of 15 % of the choline sulfate ester added.

Table II

Organism	Catalogue No.*	Weight of dry mycelium (mg.)	Incubation period (days)	Utilization of choline sulfate: Sulfate utilized ($BaSO_4$ mg./flask)	Utilization of choline sulfate: %	Weight of dry mycelium (mg.)	Incubation period (days)	Utilization of inorganic sulfate: Sulflate utilized ($BaSO_4$ mg./flask)	Utilization of inorganic sulfate: %
Neurospora crassa	6178	93	8	3.2	5.5	117	8	7.1	12.2
N. sitophila	6070	298	8	6.2	10.7	353	8	12.1	20.9
Alternaria tenuis	4026	347	9	19.7	34.0	750	9	19.3	33.3
Botrytis cinerea	5964	934	9	17.4	30.0	656	9	17.1	29.5
Fusarium solani	5893	420	8	8.1	13.9	386	8	8.2	14.1
Oospora lactis	4597	528	8	6.6	11.4	390	8	6.4	11.0
Pullularia pullulans	4464	725	8	14.7	25.4	368	8	5.8	10.0
Trichothecium roseum	6157	195	9	6.2	10.7	250	9	12.6	21.8

Initial amount of choline sulfate (or inorganic sulfate) in each flask; 58.2 mg. (on $BaSO_4$ basis).

Table III shows the results obtained for six species of yeasts. Each flask containing 50 ml. of M. No. 2–CS (or M. No. 2–SO_4'') with a sulfate content equivalent to 8 mg. sulfur/flask, was inoculated from a culture of yeast to be tested. All the organisms of this group, with only one exception, did not practically absorb

choline sulfate ester and, moreover, they often showed certain sign of incomplete growth as compared with that observed in normal medium. *Hansenula anomala*, however, absorbed 20 % of the choline sulfate ester given as sulfur source and showed greater increase in dry weight than that obtained in normal medium containing inorganic sulfate (M. No. 2–SO_4'').

Table III

Organism	Catalogue No.*	Weight of dry yeast (mg.)	Incubation period (days)	Utilization of choline sulfate		Weight of dry yeast (mg.)	Incubation period (days)	Utilization of inorganic sulfate	
				Sulfate utilized ($BaSO_4$ mg./flask)	%			Sulfate utilized ($BaSO_4$ mg./flask)	%
Saccharomyces cerevisiae	0209	92	4	0.8	negligible	116	4	4.4	7.6
Saccharomyces Strain XII	2113	140	4	0.6	〃	146	4	7.4	12.7
Hansenula anomala	4540	218	5	11.3	19.5	154	5	5.6	9.7
Schizosaccharomyces octosporus	0353	8	5	0.9	negligible	42	5	5.8	10.0
Zygosaccharomyces marxianus	0219	55	5	0.7	〃	96	5	5.4	9.3
Candida tropicalis var. *japonica*	0618	145	4	1.0	〃	150	4	4.6	7.9

Initial amount of choline sulfate (or inorganic sulfate) in each flask; 58.2mg. (on $BaSO_4$ basis)

Table IV contains the results obtained for five species of moulds belonging to Phycomycetes. Each flask containing 50 ml. of M. No. 2–CS (or M. No. 2–SO_4'') with sulfate content equivalent to 8 mg. sulfur/flask, was inoculated and incubated for a requisite period of days. Of the members of this group, only *Rhizopus oryzae* utilized choline sulfate ester as sulfur source and showed favorable growth. *Rhizopus nigricans* and *Mucor mucedo* did not utilize the ester and the growth in CS-medium insignificant. *Phycomyces* and *Absidia* failed to grow even in normal medium.

Table IV

Organism	Catalogue No.*	Weight of dry mycelium (mg.)	Incubation period (days)	Utilization of choline sulfate		Weight of dry mycelium (mg.)	Incubation period (days)	Utilization of inorganic sulfate	
				Sulfate utilized ($BaSO_4$ mg./flask)	%			Sulfate utilized ($BaSO_4$ mg./flask)	%
Rhizopus nigricans	5411	60	8	0.5	negligible	207	5	4.7	8.1
Rhizopus oryzae	4746	300	9	5.5	9.5	337	9	9.2	15.8
Mucor mucedo	5776	58	8	0.6	negligible	134	8	4.5	7.8
Phycomyces nitens	5694	90	9	0.4	〃	98	9	4.2	7.2
Absidia glauca	4002	45	9	0.5	〃	56	9	3.0	5.2

Initial amount of choline sulfate (or inorganic sulfate) in each flask; 58.2mg. (on $BaSO_4$ basis)

Table V shows the results obtained for four species of Basidiomycetes. Each flask containing 50 ml. of M. No. 3-CS (or M. No. 3-SO_4'') with sulfate content equivalent to 8 mg. sulfur/flask, was inoculated. None of this group ever utilized choline sulfate ester.

Table V

Organism***	Weight of dry mycelium (mg.)	Incubation period (days)	Utilization of choline sulfate		Weight of dry mycelium (mg.)	Incubation period (days)	Utilization of inorganic sulfate	
			Sulfate utilized ($BaSO_4$ mg./flask)	%			Sulfate utilized ($BaSO_4$ mg./flask)	%
Polystictus hirsutus	140	13	0.5	negligible	115	13	7.2	12.4
Fomes fomentarius	72	13	0.4	〃	50	13	8.3	14.4
Schizophyllum commune	56	13	0.7	〃	36	13	4.7	8.0
Pleurotus japonicus Kawam.	83	13	0.5	〃	65	13	9.2	15.8

*** Obtained from the Faculty of Agriculture, Gifu University.
Initial amount of choline sulfate (or inorganic sulfate) in each flask; 58.2mg. (on $BaSO_4$ basis)

Discussion[9]

As is generally known, most micro-organisms are capable of utilizing inorganic sulfate as sulfur source. Cowie *et al.*[7] considered that, for the case of *Escherichia coli*, SO_4'' absorbed by the organism is reduced to SO_3'', prior to the ensuing conversion into organic S-compound. The reaction scheme assumed by these authors is as follows:

$$
\begin{array}{ccccccccc}
SO_4'' & \longrightarrow & SO_3'' & \longrightarrow & \text{cysteine} & \longrightarrow & \text{homocystine} & \longrightarrow & \text{methionine} \\
 & & & & | & & & & | \\
 & & & & \text{protein} & & & & \text{protein}
\end{array}
$$

In the present study, it was revealed that among twenty-nine fungal organisms examined, six belonging to Aspergillales, two belonging Pyrenomycetes together with six Fungi Imperfecti are capable of utilizing choline sulfate ester as sulfur source. With other organisms, including five species of yeasts, four Phycomycetes, and four Basidiomycetes, the results were negative in this respect.

The above-mentioned positive results suggest a possibility that choline sulfate ester represents a normal intermediary of sulfur assimilation in these organisms, especially when we remember the fact that the substance has been identified as a normal constituent of certain fungal organisms[2)5)6)]. Although a sufficient body of evidence has not yet been obtained concerning the precise mechanisms of the process involved, the above-described finding with *Alternaria tenuis*, will be of special interest in this connetion. In one experiment with this mould, a certain quantity of sulfate ion arising most plausibly from partial hydrolysis of choline sulfate ester, was detected in the residual culture medium, while the result was negative

in this respect in another experiment performed with the same organism. The discrepancy between these two results may be due to the differences in the relative levels of activity of the organism in hydrolyzing the ester and absorbing sulfate ions, as influenced by cultural conditions. In fact, the former experiment was carried out with an ordinary flask culture, while the latter was done with a shaking culture of the same organism. A detailed study pertaining to this point is being carried out.

Most of the yeast species examind in this study, including two *Saccharomyces*, one each of *Schizosaccharomyces*, *Zygosaccharomyces* and *Candida*, showed no utilization of choline sulfate ester in the course of metabolism (see Table III). Accordingly, choline sulfate ester does not appear to be an intermediate substace of metabolism for the sulfur assimilation reaction in these organisms. However, *Hansenula anomala* which is taxonomically closely related to *Saccharomyces*[8], not only absorbed the organic sulfate ester, but showed much better growth compared with the case in which inorganic sulfate was given as sulfur source. This fact suggests that there must be some yeasts in which choline sulfate ester may be an intermediary metabolite of sulfur assimilation.

Table IV contains the results obtained for five species belonging to Phycomycetes. For *Mucor mucedo*, *Phycomyces nitens* and *Absidia glauca* did not utilize choline sulfate ester as sulfur source but, in *Rhizopus oryzae* about 9 % of choline sulfate added was consumed with a fairly abundant growth. *Rhizopus nigricans*, on the other hand did not utilize the organic sulfate ester.

It may be inferred from these findings that the capacity for utilizing choline sulfate ester is not limited to the class of Ascomycetes, in which the substance has been discovered as a normal constituent, but is of more or less common occurrence also among Phycomycetes and Fungi Imperfecti, in which the production of the substance has not yet been reported.

It may be of some interest to note in this connection that all of the four higher fungi (Basidiomycetes) examined in the present study showed more favorable growth in the presence of choline sulfate ester (M. No. 3–CS) than in the sulfate medium (M. No. 3–SO_4''), although there was no appreciable consumption of the added choline sulfate ester during the incubation period. This fact seems to indicate a possibility that choline sulfate ester may be playing the role of a growth facter in Basidiomycetes, although the final decision must await further investigation.

Summary

The utilizability of choline sulfate ester as sulfur source was examined in twenty-nine fungi belonging to the following Classes: Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti.

Choline sulfate ester was found to be utilized as sulfur source in Aspergillales,

Pyrenomycetes and Fungi Imperfecti. In some cultures with *Alternaria tenuis* an accumulation of certain amounts of sulfate ions in the residual culture medium was detected.

No utilization was revealed in yeasts (*Saccharomyces*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Candida*), a single exception being *Hansenula anomala* which consumed the substance to give a fairly abundant growth in its presence in the culture medium.

Choline sulfate ester was not utilized in the Phycomycetes species studied (*Rhizopus nigricans*, *Mucor*, *Phycomyces*, *Absidia*), with the single exception of *Rhizopus oryzae*, which utilized the substance to show a fairly good growth in its presence.

All the Basidiomycetes species examined did dot appreciably consume choline sulfate ester but all of them showed enhanced growth in the presence of this substance.

In conclusion the author wishes to express her sincere appreciations to Prof. F. Egami (University of Tokyo) and Prof. T. Mori (Nagoya University) for their kind guidance throughout the investigation and for their kindness in reading the original manuscript.

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摘　　要

藻菌類，嚢子菌類，担子菌類および不完全菌類の4つの綱に属する菌類29種について，硫黄源としてのコリン硫酸エステルの効用をしらべた。

Aspergillales, Pyrenomycetes および不完全菌類はいずれもコリン硫酸エステルを硫黄源としてよく利用する。*Alternaria tenuis* の培養液中にはコリン硫酸エステルの加水分解によつて生じた硫酸イオンがみられた。

酵母菌類はコリン硫酸エステルを利用しないものが多い (*Saccharomyces*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Candida*) が，*Hansenula anomala* だけがこれを硫黄源としてよく発育した。

藻菌類はコリン硫酸エステルを利用しないものが多い (*Rhizopus nigricans*, *Mucor*, *Phycomyces*, *Absidia*) が，*Rhizopus oryzae* だけがこれを硫黄源として利用し，よい発育を示した。

担子菌類はいずれもコリン硫酸エステルを硫黄源として利用しないが，これを与えた場合は正常培地におけるよりも一般によい発育を示した。

On *Streptomyces massasporeus* nov. sp.*,**

by Ryuji SHINOBU*** and Mineko KAWATO***

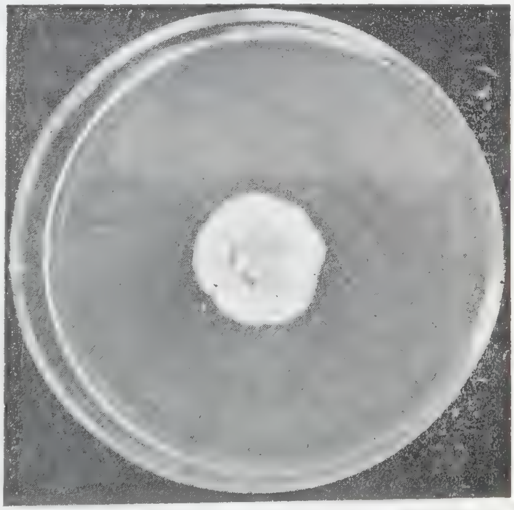
信夫隆治***・川戸峰子***: 新種 *Streptomyces massasporeus* について

Received March 5, 1959

The strain, No. 602, isolated from the soil collected at Doro, Wakayama Prefecture in October 1957, was identified to be a new species when compared carefully on the morphological and physiological characteristics with the species described so far, and it was named '*Streptomyces massasporeus* nov. sp'. The characteristics of this species will be given in detail in the following.

I. Morphological Characteristics

Czapek agar, ammonium Czapek agar, glucose asparagine agar (Krainsky's), Ca-malate agar, starch agar (Waksman's[2] and Masumoto's[3]), and glycerine starch glutamine agar[1], etc. were used for the morphological observation. For the microscopical study, glucose asparagine agar was mainly used, because more aerial mycelium was formed on this medium than on the others, showing its characteristics of the aerial mycelium, for example, typical spirals. For the purpose of observation, colonies were incubated for 5-10 days in 28-30°.



9 cm

Photo. 1. Macrocolony, incubated for 20 days in 28-30° on glucose asparagine agar; Chrysanthemum pattern.

1. Macrocolony
   Chrysanthemum pattern on glucose asparagine agar as seen in Photo. 1.
2. Microscopical observation
   Aerial mycelium: Branching, monopodial; Anitella type whirls[1] and tufts were occasionally seen, though they were not remarkable; the width of the aerial mycelium, about

* The outline of this study was already reported at the 23rd General Meeting of Botanical Society of Japan (1958).

** As for the descriptive method of species, that of Shinobu's method[1] was adopted.

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0.8 μ.

Spiral: Numerous; compact and short (2-3 turns) and loop-like spirals were comparatively dominant; sometimes long and compact or long and loose spirals (5-6 turns); seldom irregular one; diameter of spirals, usually 3-4 μ; sinistrorse.

Conidia: Spherical or elliptical; about 0.8 μ in length. Conidia formation began in 2-5 days' cultivation.

A B

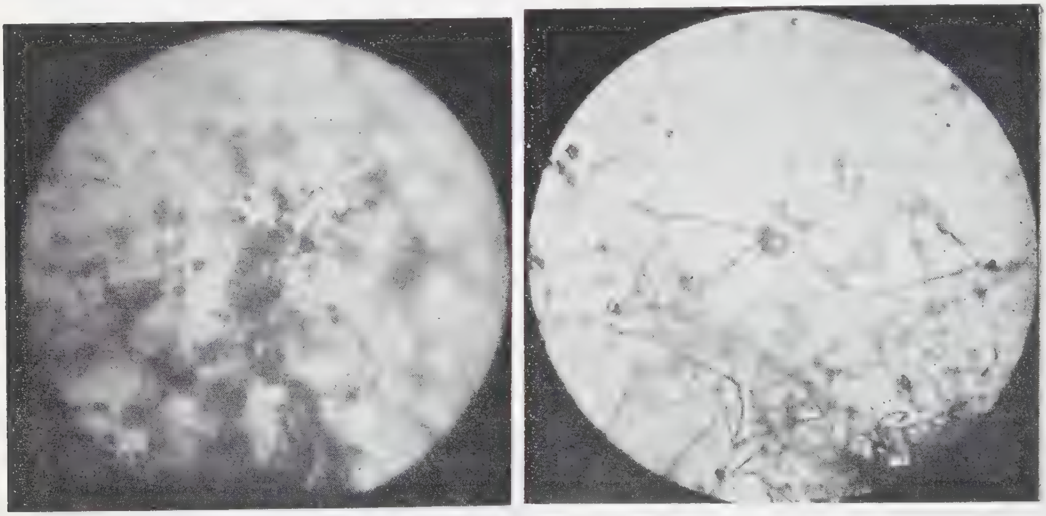


Photo. 2. Spore mass formation, incubated for 8 days on glucose asparagine agar.
A: Spore masses B: Magnification of spore mass

Many spherical masses of spores could be seen in the colony as shown in Photo. 2. Spore mass formation was remarkable on glucose asparagine agar and Ca-malate agar; but not remarkable on the other media.

Diameter of spore masses: 3-4 μ at the least; the larger it became, the more irregular it shaped.

II. Physiological Characteristics

1. Tyrosinase reaction: Positive and remarkable.
2. Nitrite production: Positve on modified synthetic solution.
3. Diastase reaction by the iodine test: Weak—moderate.

   Enzymatic zone: $\left\{\begin{array}{l}3\pm2\text{ mm. on glycerine starch glutamine agar}\\ 6\pm2\text{ mm. on starch agar (Waksman's A)}\end{array}\right\}$ incubated for 7 days.
4. Konjakmannase reaction: Weak.
5. Utilization of C-sources: Xylose, rhamnose, sucrose, fructose, raffinose, mannitol and lactose were utilized.

III. Cultural Characteristics

Notes:
G........................growth of colony.
A........................formation of the aerial mycelium.
S........................color of the aerial mycelium.
P........................production of the soluble pigment.

1. Ammonium Czapek agar
   G: moderate—poor; thin.
   A: spur—none; only at the margin of the colony; brownish white.
   S: pale yellow orange—pale yellow orange with pinkish tinge.
   P: none.
2. Glycerine Czapek agar
   G: good.
   A: good; sometimes poor; purplish gray—pale purple.
   S: deep purple—brown purple.
   P: pale purple with brownish tinge—pale brown.
3. Glucose asparagine agar
   G: good; sometimes intruded deep into medium.
   A: good; light purplish gray—pinkish gray—reddish gray.
   S: dull red purple—dark red purple.
   P: pale grayish red brown—pale dark red purple.
4. Ca-malate agar
   G: good—moderate.
   A: good—moderate; somewhat thin; pinkish gray—light purplish gray.
   S: brown purple—grayish red purple—brown.
   P: uncertain; very pale brown, if any.
5. Starch agar (Waksman's A)
   G: good—moderate; somewhat thin.
   A: moderate; sometimes poor; pinkish gray.
   S: grayish purple—pale dull purple.
   P: uncertain; very pale grayish purple or pale dull pink, if any.
6. Starch agar (Masumoto's)
   G: good; intruded into medium.
   A: moderate; somewhat thin; Rose Dust—pinkish gray.
   S: dull red purple—red—dull red; sometimes intruded mycelium showed vein-like growth on account of the production of red or dull red pigments.
   P: none.
7. Urea glycerine agar[1)]
   G: good.
   A: moderate—poor; partial; sometimes only at the upper side of the colony.
   S: brown purple—brown.

P : uncertain; probably, none.

8. Glycerine starch glutamine agar

G : good.

A : good—moderate; sometimes partial; light brownish gray—pinkish gray—purplish gray.

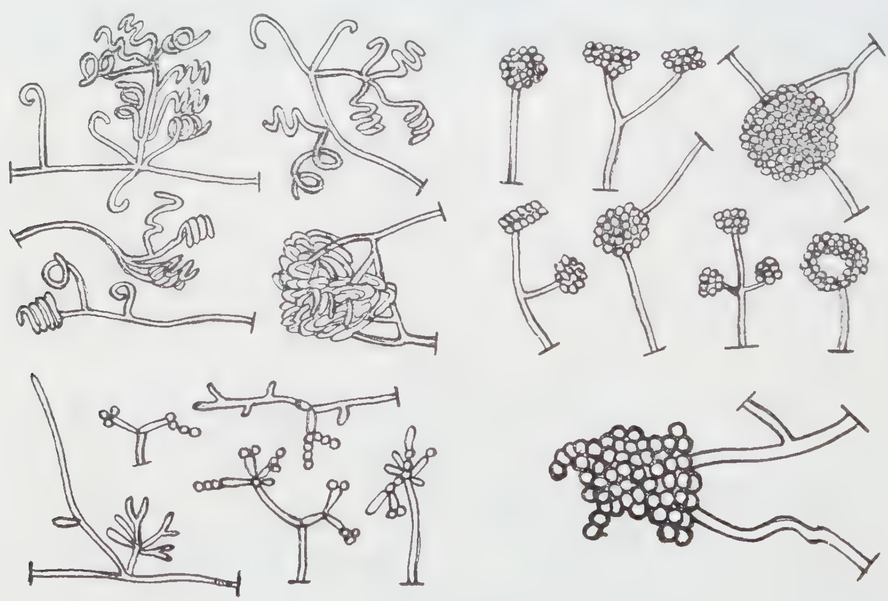
S : brown purple—grayish purple.

P : pale brownish purple.

IV. Consideration and Conclusion

To summarize the results of the morphological observation, the branching of this strain was generally monopodial; but Anitella type whirls and tufts were occasionally seen on the aerial mycelium, though they were not remarkable. Many various kinds of spirals were formed on glucose asparagine agar and other media. Sometimes, two or three spirals touched, or tangled with, each other at the ends of spirals as seen in Fig. A and D. This strain produced a quantity of tiny droplets on glucose asparagine agar and on other media as the aerial mycelium grew, and showed so-called 'wetted type'. Spores had a tendency to diffuse easily. Spores

A B



C D

Fig. Sketch of spiral and spore mass

A : Ordinary spirals and tangled ones.

B : Spore mass formation.

C : Special conidia formation could be seen also on the skirt of the colony: though seldom.

D : Magnification of spore mass formation after tangling of spirals.

at the neighborhood of droplets were secondarily formed into a mass of spores due to the surface tension of droplets, and showed a remarkable characteristic as shown in Fig. B. The larger the mass of the spores grew, the more difficult it became to keep its spherical shape, and at last it touched the substrate or other droplets, and expanded irregularly.

Now, besides the media described above, many media, i. e. egg, carrot, potato, Bouillon, broth, gelatin and milk etc. were also used for the study of the physiological and cultural characteristics. These characteristics were summarized as below.

As this strain produced water soluble and brown pigments on most of organic media, though some of them showed purplish or reddish tinge, this was one of a 'Chromogenus species'[5]. Moreover, considering the colors of the aerial mycelium which showed purplish—pinkish on many media, the authors would like believe that this strain belongs to '*Streptomyces lavendulae* Group'[6].

On Masumoto's starch agar, the substrate mycelium intruded deep into the medium, and produced insoluble red pigment. It accumulated partially in the substrate mycelium and looked like a blood vein on observation through the test-tube, which was a remarkable characteristic. When these characteristics mentioned above are compared with those of the species[6),7] reported so far, following three species prove to resemble each other.

Str. lavendulae (Waksman and Curtis) Waksman and Henrici[8),9].

Str. virginiae Grundy, Whitman, Rdzok, Rdzok, Hanes, and Sylvester[10].

Str. venezuelae Ehrlich, Gottlieb, Burkholder, Anderson, and Pridham[11),12].

Str. venezuelae does not form any spiral, while No. 602 forms many of them. And there can be seen many differences of cultural characteristics between No. 602 and *Str. venezuelae*. Consequently, No. 602 is one species and *Str. venezuelae* is another.

Table

Medium / Strain	Production of soluble pigment		
	No. 602	*Str. lavendulae*	*Str. virginiae*
Synthetic agar	+	—	—
Glucose asparagine agar	+	—	—
Nutrient agar	+	+	—*
Glucose broth	+	—*	+
Nitrite production	positive	positive	none—limited

* The type cultures of *Str. lavendulae* and *Str. virginiae* used for the comparison with No. 602, were given by Institute for Fermentation of Takeda Pharmaceutical Co. and Shionogi Pharmaceutical Co. and they showed positive results in the author's experiment, though negative in the original description[9),10].

Next, the differences of cultural and physiological characteristics between No. 602, *Str. lavendulae* and *Str. virginiae*, are shown in the table.

Moreover, neither of *Str. lavendulae* and *Str. virginiae* produced any spore mass, nor showed any vein-like growth. According to the experiments stated above, No. 602 was decided to be a new species and it was named '*Streptomyces massasporeus*'. The specific epithet means that this species forms many spherical masses of spores.

Acknowledgement

The authors should like to express their hearty thanks for Dr. Teijiro Kishitani and Dr. Yasona Fukuda, who kindly gave them helpful suggestions on this article. They also express their hearty gratitude for Takeda Pharmaceutical Co. and Shionogi Pharmaceutical Co., that sent them type cultures of *Str. lavendulae* and *Str. virginiae*.

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摘　　要

1957年10月，和歌山県瀞の土から分離された放線菌の一菌株 No. 602 を形態学的ならびに生理学的に従来記載された種と慎重に比較検討を行なつた結果，新種と断定したので，この菌株が空中菌糸に円形の胞子塊 (Spore mass) を形成することを種名にとり入れて *Streptomyces massasporeus* と命名した。

A Cytochemical Study on the Proteid Vacuoles in the Egg of *Pinus thunbergii* Parl.

by Akio TAKAO*

高尾昭夫*: クロマツの卵細胞内に存在する蛋白胞の細胞化学的研究.

Received March 16, 1959

In the egg cell of conifers, a kind of peculiar cellular organelles was found in the cytoplasm by Hofmeister[1] and they have been called with his name, "Hofmeister's bodies". About the nature of these structures various opinions have been presented by many investigators. These opinions may be ranked in following two lines: the first is that these structures are derived from the nuclei of jacket cells (Goroshankin[2], Arnoldi[3] etc.), and the second is that they are a type of vacuoles. The latter, however, has been rather more generally accepted. Also, Strasburger[4] called them "proteid vacuoles", observing the coagulated artefacts of them by alcohol. Furthermore, Stopes and Fujii[5] reported that these structures contained proteins and took part in nutrition of the egg cell. Also, Ferguson[6] called them "nutritive spheres".

In this paper, the cytochemical studies on the proteid vacuoles of the egg cell of *Pinus thunbergii* are reported.

Material and Methods

The cones of *Pinus thunbergii* Parl. pollinated in the previous year were collected from the field of the Faculty of Science of Nagoya University from the middle to the end of June in 1955 and in 1956.

The ovules taken out from the cones were fixed in the following fixatives: Telyesniczky's, Bouin's, ethanol-$HgCl_2$ (50% ethanol 100 ml., glacial acetic acid 10 ml. and mercuric chloride 8 g.), 10% formalin and ethanol-formalin (absolute ethanol 90 ml., formalin 10 ml. and glacial acetic acid several drops). After fixation the ovules were dehydrated through a graded series of ethanol and were embedded in paraffin by usual method. Then they were longitudinally sectioned at a thickness of 10 μ.

Heidenhain's iron-hematoxylin, toluidine blue or methyl green-pyronin were used for staining. Lillie's method was employed for staining polysaccharides in general (Lillie[7]). Iodine-potassium iodide solution was applied to detect starch. For staining proteins, ninhydrin-Schiff reaction (Yasuma and Ichikawa[8]), Serra's method for ar-

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ginine and arginine-containing proteins (Serra[9]) and Millon's reaction by Bensley's prescription (Lison[10]) were employed.

On some of the cones, centrifuge treatments were carried out. After centrifugation at 1000 g to 1600 g for 30 minutes, the ovules were taken out from the cones and fixed by the above-mentioned fixatives.

Results

Before fertilization.

In the central cell, shortly after the neck cell has been cut off, there is a little amount of cytoplasm, for many vacuoles (in general sense) of varying size are occupying nearly all parts of the cell. While the cytoplasm increases more and more, the vacuoles (in general sense) begin to disappear from the lower and the peripheral parts of the cell. At first the cytoplasm is seen to be uniform and is stained faintly with ninhydrine-Schiff, Millon's and arginine reactions and Heidenhain's iron-hematoxylin. With the disappearance of the vacuoles (in general sense), a few granular organelles appear from the lower and the peripheral parts or sometimes among vacuoles (in general sense) in the cytoplasm (Fig. 1). These granular organelles are different from cytoplasm in the stainability, notwithstanding their border lines are still indistinct. Also these organelles assume faintly orange, pink and pale red colors with arginine, Millon's and ninhydrine-Schiff reactions, respectively. In early stages these organelles are characterized by staining with ninhydrine-Schiff reaction (Fig. 7). While in a somewhat later stage by staining with arginine and Millon's reactions. These organelles develop to give rise to the proteid vacuoles.

Just before and after the egg nucleus is formed, the proteid vacuoles have been scattered all over the cytoplasm of the cell with disappearance of the vacuoles (in general sense). The proteid vacuoles are still hazy and stained somewhat weakly by Heidenhain's iron-hematoxylin (Fig. 2) with arginine, Millon's and ninhydrine-Schiff reactions. On the other hand, the cytoplasm in the egg cell becomes more and more densely stained and assumes a relatively strong color with Heidenhain's iron-hematoxylin and protein staining until egg nucleus is formed. In the protein staining the colors of the cytoplasm are orange, red purple and pale red or yellow respectively by arginine, ninhydrine-Schiff and Millon's reactions.

When the egg nucleus migrates toward the center of the cell with enlargement of it's size, the proteid vacuoles tend to increase in number; they are more strongly stained by Heidenhain's iron-hematoxylin (Fig. 3) and protein staining reactions (Fig. 8), their border lines become distinct. This condition continues until just before fertilization. Within the proteid vacuoles there are some small granules, which are intensely stained with Heidenhain's iron-hematoxylin (Figs. 3 and 4). Each granule, moreover, consists in one or more very small granules, which are stained much more intensely by Heidenhain's iron-hematoxylin. From the time just before

or after fertilization, the proteid vacuoles tend to become hazy in shape and gather toward the lower part of the cell (Fig. 4).

After fertilization

The fertilized egg nucleus performs two successive divisions in the center of the egg cell resulting in four nuclei. Each of these nuclei becomes to be enveloped with new cytoplasm, which is different from the older egg cytoplasm in its uniformity in appearance. This new cytoplasm is stained distinctly with arginine, Millon's and ninhydrine-Schiff reactions (Figs. 10 and 11) and Heidenhain's iron-hematoxylin (Fig. 17), whereas the older cytoplasm is stained somewhat faintly as its density declines. In this stage, the proteid vacuoles begin to disappear with decrease in their stainability from the upper and the peripheral region of the egg cell. Thus, the proteid vacuoles remain only in the lower part of the egg cell. Though their border lines become to be more or less indistinct, their stainability is stronger than that before this stage (Figs. 5 and 11).

The four nuclei descend to the base of the egg and give rise to a four-celled proembryo. Thereafter, by two successive divisions these four cells result in 16 cells consisting of 4 tiers, each of them having 4 cells. In the 4-celled proembryo, the cytoplasm is stained very intensely with Heidenhain's iron-hematoxylin, pyronin, toluidine blue and with protein-staining reactions. In the following stage the daughter cells have similar tendency to that mentioned above, while their sizes become much smaller than in the previous stages (Fig. 6). On the other hand the egg cell gradually loses its cytoplasm and assumes faint colors with various staining reactions. Such an egg cell has little proteid vacuoles (Figs. 6 and 12).

The second tier from the base, the suspensor tier, begins to elongate and pushes out the tip cells into the endosperm tissues. The cells of this tier continue to elongate, and development of the embryo is achieved by successive divisions of the tip cells.

When the proteid vacuoles are stained by basic dyes, they show a red-violet color by toluidine blue and a red by pyronin. The stainability with these dyes is constant during all the stages observed. By Lillie's polysaccharide staining the proteid vacuoles show a pink color and by I_2-KI solution they are not stained throughout all the stages.

Centrifuge treatment

By a centrifugal force of 1000 *g* to 1600 *g*, a stratification of the cellular contents is brought about. When a central cell is centrifuged, the nucleus and a layer of cytoplasm intensely stainable by Heidenhain's iron-hematoxylin is sedimented at the centrifugal end of the cell. Over this layer, a layer of weakly stainable cytoplasm and a layer of the vacuoles (in general sense) are successively heaped (Fig. 13).

In the case of the egg cell, the arrangements of the cellular contents under a

centrifugal force show three different features in the process of egg development. In the early stage, the cytoplasm is gathered at the centrifugal end, the nucleus lying over it, and the proteid vacuoles are at the centripetal end (Fig. 14). In the maturation stage before fertilization, the proteid vacuoles are parted toward two different directions by the condition whether their contents are rich or poor. Therefore, some proteid vacuoles having rich contents are heaped on to the centrifugal end and the others having poor contents to the centripetal. Those with intermediate amounts of contents remain in the intermediate region (Fig. 15). It is worthy of interest that the fertilized nucleus assumes the least specific gravity in the cell just after fertilization, and moved to the centripetal end (Fig. 16). The proteid vacuoles are heaped at the centrifugal end in this stage.

In the division stage of the fertilized nucleus, again the divided nuclei and the remaining proteid vacuoles are thrown down to the centrifugal end by centrifugal force (Fig. 17). The divided nuclei are always accompanied by some cytoplasmic envelopes which are intensely stained by Heidenhain's iron-hematoxylin (Fig. 17) and by staining reactions for proteins.

Discussion

In *Pinus cembra*, *P. montana* and *P. sylvestris*, Stopes and Fujii[5] reported that the proteid vacuoles were observed after the egg nucleus was cut off from the central cell, and remained till the stage of suspensor elongation. In *Pinus thunbergii*, before the egg nucleus is cut off from the central cell, the initials of the proteid vacuoles are recognized by the staining reaction, notwithstanding their border lines were still more or less indistinct. Considering from these facts, it may be said that Stopes and Fujii[5] did not observe the initials of the proteid vacuoles but the fully developed shape of them. Furthermore, in *P. thunbergii* little proteid vacuoles but the empty ones or ones poor in substances are found after the formation of proembryo at the base of egg cell. Thus, in these stages they appear to become to function.

It has been reported by Strasburger[4] and many investigators after him that the proteid vacuoles contained proteins. In the present study on *P. thunbergii*, the proteid vacuoles are stained strongly by ninhydrin-Schiff and arginine reactions. It may be concluded, therefore, that large amounts of basic proteins containing arginine exist in the proteid vacuoles, while there are a little tyrosine-containing proteins, because the staining with Millon's reaction is weak. Furthermore, Stopes and Fujii[5] reported the existence of starch grains in the proteid vacuoles. In *Pinus thunbergii*, on the contrary, there is no starch but a little amount of other polysaccharides in homogeneous state.

Judging from the facts that those proteid vacuoles in which there are only a little contents tend to be shifted toward the centripetal end by the centrifuge treat-

ment, it is possible that the specific gravity of these vacuoles is small; they may contain lipids. To my regret, this possibility cannot be confirmed in the present study.

Thus, the "proteid vacuoles" named by Strasburger[4] are revealed to contain PNA, polysaccharides and possibly lipids as well as proteins. They were also called "nutritive vacuoles" by Stopes and Fujii[5]. It seems that these names are based on the various substances, which are included in the vacuoles and are used for oogenesis. In this study it can be considered that the proteid vacuoles store various substances and serve as nutritives for egg cell. In *Pinus thunbergii*, the egg cell in the maturation stage is full of proteid vacuoles. Considering their decrease in number till the fertilized egg begins to divide, the function of the proteid vacuoles may be related to the fertilization. Furthermore in an early stage of the proembryo development, many proteid vacuoles stained strongly by protein-reactions gather on the base of the egg cell and disappear with elongation of the suspensor. Judging from these facts it may be that the contents of the proteid vacuoles are consumed for development of the proembryo and elongation of the suspensor.

Many proteid vacuoles include some granules, but sometimes there are a few proteid vacuoles which have only one granule in each. In materials fixed by formalin (Figs. 9 and 10) and in a photograph of the egg cell fixed by Champy's fluid (Shimamura[11], Fig. 3), the proteid vacuoles are shown to be more or less homogeneous. In his study on the fresh eggs of *P. sylvestris*, Zacharias[12] observes the proteid vacuoles in which a homogeneous and indistinct structure and small spaces are present. The proteid vacuoles *in vivo* may have homogeneous structure, that is, some granules in them may be a kind of artefacts by fixation.

Summary

1. The "proteid vacuoles" in the cytoplasm of egg cell of *Pinus thunbergii* were studied with staining methods.

2. The proteid vacuoles first appear in the central cell. With maturation of the egg cell, they increase more and more in number and in their stainability by Heidenhain's iron-hematoxylin and protein-staining reactions. The number and the stainability of them reach the maximum state just before fertilization. After fertilization they begin to decrease in number and nearly completely disappear by the time of proembryo formation at the base of the egg cell.

3. The proteid vacuoles contain not only protein but also PNA and a little polysaccharides.

4. In all the stages of oogenesis, the proteid vacuoles are separated into three groups by centrifuge treatment; the first of them are moved toward the centrifugal end, the second toward the centripetal end and the remaining ones are distributed in the middle part of the cell. Those gathered at the centripetal end under centri-

fugal force are probable to contain lipids.

5. The nutritive significance of the proteid vacuoles in the maturation of egg cell and in the early development of proembryo is discussed.

The writer wishes to express his deep sense of gratitude to Professor Dr. T. Shimamura under whose kind guidance and encouragement this work has been carried out. He also wishes to express his thanks for Mr. T. Ôta for his helpful advice and criticism.

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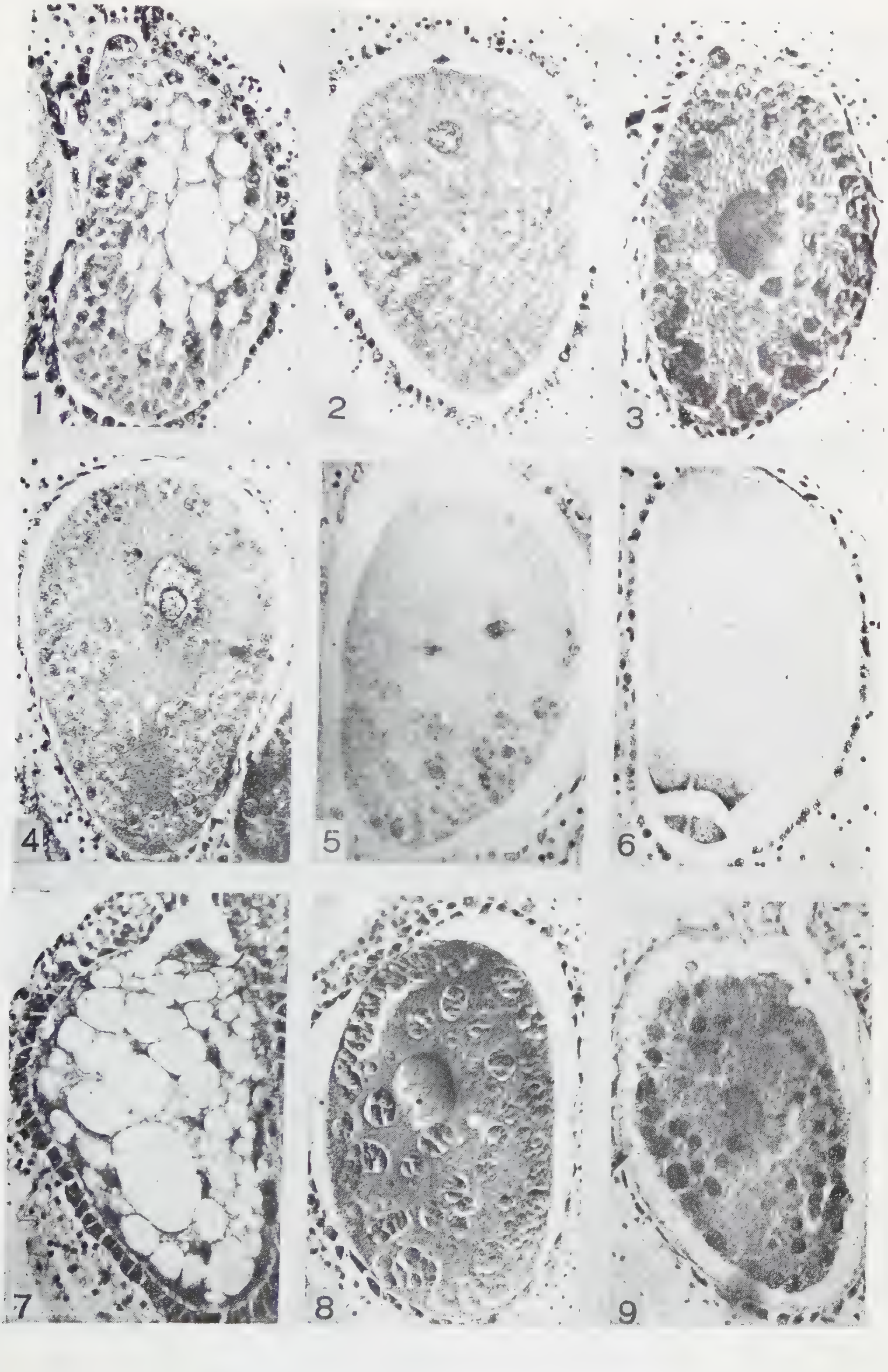
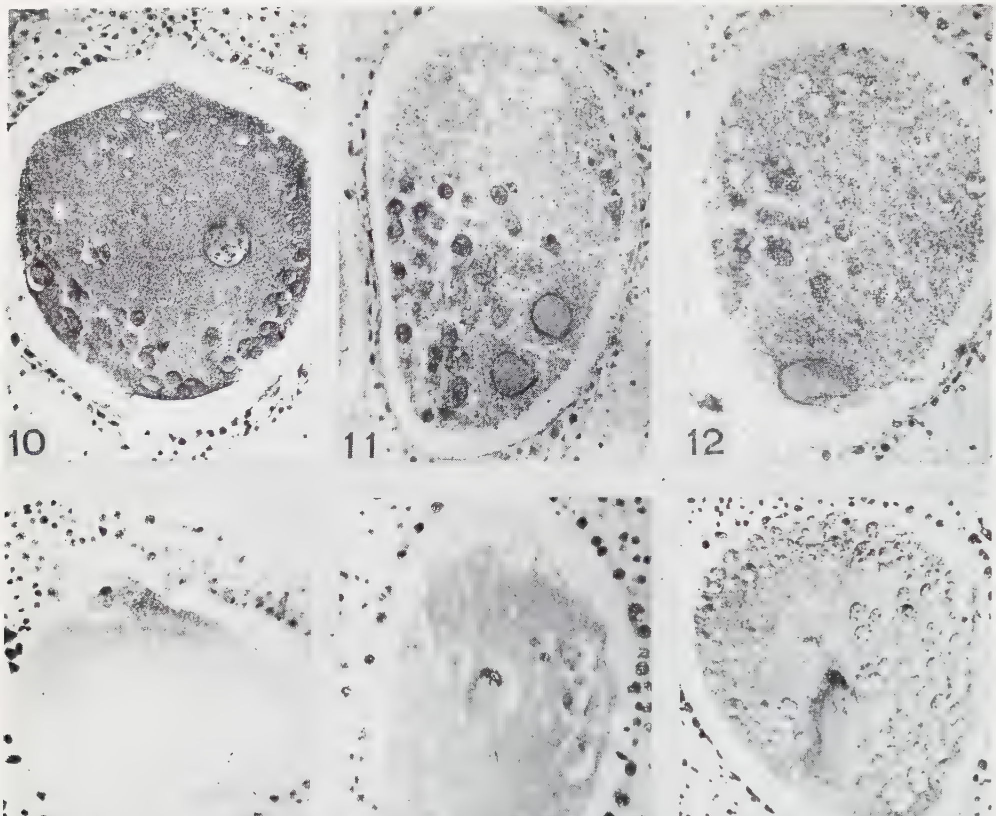
Explanation of Figures

Figs. 1—6. Egg cells and proembryos stained by Heidenhain's iron-hematoxylin. ca. ×110. Fig. 1. Central cell. With disappearance of the vacuoles (in general sense), a few granular organelles (initials of the proteid vacuoles) appear in the lower and the peripheral parts of the cell. Fig. 2. Immature egg cell. While the vacuoles (in general sense) disappear completely, the proteid vacuoles are still hazy. Fig. 3. Before fertilization. The proteid vacuoles reach the maximum in number. Fig. 4. Nuclear fusion. The proteid vacuoles become hazy in their shape and gather toward the lower part of the cell. Fig. 5. The second mitosis after fertilization. There are the proteid vacuoles in the lower part of cell. Fig. 6. 8-celled proembryo. Little proteid vacuoles are seen. Figs. 7—12. Egg cells and proembryos stained with ninhydrin-Schiff reaction. ca. ×110. Fig. 7. Central cell. The granular organelles (the initials of the proteid vacuoles) in the lower part of the cell and among the vacuoles (in general sense) is strongly stained. (cf. Fig. 1, which shows the later stage than that in Fig. 7). Fig. 8. Mature egg cell. The proteid vacuoles still do not reach the maximum in number. Fig. 9. Fertilized egg cell. The proteid vacuoles begin to decrease in number.

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Figs. 10 and 11. 4-nucleated proembryo (not all of the four nuclei are seen in this picture). The proteid vacuoles gather in the lower part of the cell. Fig. 12. 4-celled proembryo (only one of the four nuclei is seen). The proteid vacuoles are degenerating. Figs. 13—17. Centrifuged egg cells and proembryos stained by Heidenhain's iron-hematoxylin. (the arrows show the direction of the centrifugal force applied.) Fig. 13. Central cell. The nucleus, a layer of strongly stainable cytoplasm, a layer of weakly stainable cytoplasm and a layer of the vacuoles (in general sense) are successively heaped. Fig. 14. Immature egg cell. Most proteid vacuoles gather at the centripetal end. Fig. 15. Before fertilization. The proteid vacuoles are parted toward two opposite directions. A few proteid vacuoles remain in the intermediate region. Fig. 16. After fertilization. The proteid vacuoles are heaped at the centrifugal end, while the nucleus are thrown down to the centripetal end. Fig. 17. 4-nucleated proembryo. The nucleus and remaining proteid vacuoles are thrown down to the centrifugal end.

摘　　要

[illegible]

[illegible]白胞は中心細胞の時期に現われはじめる。腹溝細胞が形成され，卵細胞が成熟するにつれて蛋白胞は数を[illegible]前にその数は最大に達し，[illegible]胞全体にみられるようになる。受精後は蛋白胞の数は減りはじめ，卵細胞の底部で前胚形成が進む時期までには殆んど完全になくなる。

[illegible]ばかりでなく PNA の少量，多糖[illegible]も考えられる。

[illegible]

The Species of *Carex* in the Himalayan-Japanese Link
Taxonomic study of Cyperaceae 10**

by Tetsuo KOYAMA*

小山鐵夫*: ヒマラヤ日本要素のスゲ類

Received March 18, 1959

§22. A group of Japanese plants called the Himalayan-Japanese species is an example of a disjunct distribution. As illustrated in Fig. 21, the main area of such plants spreads widely over the highland of Himalaya with very isolated small areas in Japan and its surrounding regions. The common geographical characteristic of these plants is that they are quite absent from central and northern Chinese lowlands. The main area usually covers all over the Himalaya Mountain Range from Kashmir westwards to Afghanistan, or further eastwards to Yunnan and Szetchuan in China. Rather in many cases, the high mountains of Ceylon, the southern part of Deccan Peninsula, west coast of Sumatra, and Java are also marked as the isolated small areas. Further it is notable that the plants of the Himalayan-Japanese link are never found in the Philippines, Borneo, Celebes, and New Guinea, where they are replaced by a more common other group, the plants in the Indian-Malaysian link. Although the areas of these two different distribution types overlap one another in the southern part of India, Ceylon, Sumatra and Java, these two are generally distinct phytogeographically.

The Japanese flora being relatively well known, the data from Japan enable us to draw a fairly accurate boundary of the distribution area of each entity as for the area in Japan, while it is often very difficult to fix especially the northern and the easternmost boundaries of the main area, owing to the problems of the Himalayan Carices in Tibet left still in the vague, and to the lack of data available from the northern part of Thailand. The main area, therefore, may be actually more continuous in its southeastern end, and its northern boundary facing Tibet may extend far more northwards.

On account of its large number of species, the genus *Carex* provides us with very good materials for the phytogeographical observations in taxonomy. Among some 200 Japanese species of *Carex*, those of the typical Himalayan-Japanese link are represented by nine, viz. *C. fedia*, *C. Jackiana-parciflora* complex, *C. ligulata*, *C. maculata*, *C. nubigera*, *C. olivacea-confertiflora* complex, *C. pruinosa-Maximowiczii* complex, *C. Rochebruni*, and *C. teinogyna*. The following are the taxonomical observa-

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** Continued from Bull. Arts & Sci. Div., Ryukyu Univ. No. 3, 78 (1959).

tions made on these nine entities.

In preparing this paper, my heartfelt thanks are due to Dr. G. Taylor (Director) and Mr. E. Nelmes of Kew, for their kindness in sending me several Indian and Malaysian Carices on loan, which were indispensable to me for the final criticism of some species. The collection made by the Japanese-Himalayan Expedition and various small collections from China, both kept in the Kyoto University (KYO), and Dr. Hayata's Indo-Chinese collection in the University of Tokyo (TI) were of great use. Almost all the Far Eastern specimens used in this study are preserved in my private herbarium. I am also grateful to Professor H. Hara and Dr. J. Ohwi who are always at hand with kindness and encouragement.

(1) *Carex fedia* Nees ex Wight—Fig. 21.

Indian *C. fedia*, more commonly known as *C. Wallichiana*, has Japanese and Korean variants in the Far East often referred to var. *Miyabei* and var. *pilifera* respectively. Though rather isolated geographically, the typical var. *fedia*, var. *Miyabei*, and var. *pilifera* are strikingly akin except for a few characters mentioned in the table below.

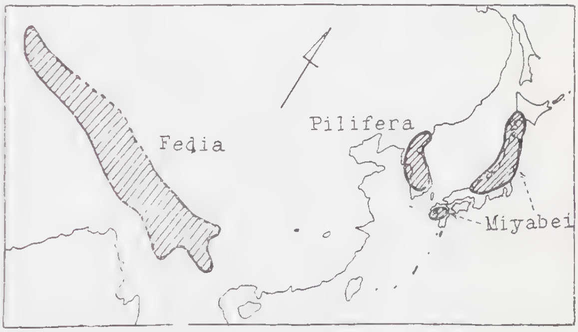


Fig. 21. Distribution of *Carex fedia* s. lat.

Comparing var. *Miyabei* with var. *pilifera*, I have a strong impression that they are independent from an evolutionary point of view, and in the general aspect var. *pilifera* comes closer to Indian var. *fedia* than to var. *Miyabei* itself

Table showing the characteristic differences among the 3 variants of *C. fedia* Nees.

Taxon / Parts	*Fedia*	*Pilifera*	*Miyabei*
Basal sheaths	coriaceous, chestnut-purple.	rigid, reddish-brown.	rigid later, reddish-brown.
Female spikelets	short-peduncled (less than 5 mm.) to sessile, the body 6-7 mm. thick with very dense flowers, the uppermost one often with staminate part.	nearly sessile or the lowest with short peduncle inclosed in bract sheath, dense-flowered, 6-7 mm. thick, sex distinct.	peduncle elongate to 2 cm., body 5-6mm. thick with flowers rather loosely disposed, sex usually distinct.
Perigynia	coriaceous, ovate, 5-6 mm. long with an upright beak about 1 mm. long, wholly very hairy thus nerves being very obscure.	subcoriaceous, ovate, 5-6mm. long, abruptly contracted to an upright beak almost 1 mm. long, very loosely hairy.	herbaceous, ovate-elliptical, 4-5 mm. long, with gradually narrowed 1.5 mm. long beak, less hairy thus faintly nerved.

except for its nearly glabrous perigynia, though on the map (Fig. 21) their areas are seen as if there were an evolutionary relationship between var. *pilifera* of Korea and var. *Miyabei* in Kyushu, Japan. However, it is natural to consider that var. *Miyabei* in northern Honshu, and western Hokkaido spread to Japan from the north, possively through Saghalien. Further a special mention is needed as to its occurrence in Kyushu. The Kyushu plants in such a pattern of distribution are usually connected with the Korean-Japanese link, however, in this sedge, no close taxonomical relationship is found between var. *Miyabei* in Kyushu and var. *pilifera* in Korea as stated above, and Kyushu is only regarded as the southernmost locality of the northern var. *Miyabei*. Accordingly, all var. *Miyabei* in Japan would have migrated into Japan only through the northern route of distribution or the socalled Saghalien-Hokkaido Stepping. Really at present, such a northern plant as *Scirpus Wichurai* is collected in northern Kyushu. Korean var. *pilifera*, by the way, seems not to have reached Kyushu, though the Kyushu individuals were once referred to *C. glabrescens*, a synonym of var. *pilifera*.

(2) *Carex pruinosa* Boott—Fig. 22.

C. pruinosa is represented in Japan by well known *C. Maximowiczii*. Up to the present the former has been known only from Himalaya and Java. The recent discovery of *C. pruinosa* from the Indo-Chinese peninsula connected the very remote two localities though incompletely, suggesting that the distribution pattern of *C. pruinosa* might come close to that of *C. Maubertiana* (cf. T. Koyama in Act. Phytotax. et Geobot. **16**: 38, f. 5. 1955).

Although *C. Maximowiczii* resembles *C. pruinosa* s. str. in the general appearance, it is fairly well differentiated morphologically from the latter, hence most specialists concerned have treated it to be specifically distinct from the latter. Kükenthal, however, was the first who found the taxonomical similarity between these two, treating *C. Maximowiczii* as a subspecies of *C. pruinosa*. Both from geographical and morphological observations, I agree with the opinion of Kükenthal that the two must have been originated from their common ancestor thought to be similar to *C. pruinosa* s. str. in the present day. The main differences between them are keyed as follows:

A. Spikelets 4 to 5 to a culm, suberect to subcoriaceous, the body 2 to 5cm. in length and 4 to 7mm. in width; perigynia elliptical 3-4mm. by 1.8-2.2mm., with an elliptical-ovate scale. .... Subsp. *pruinosa*.

AA. Spikelets 2 to 4 to a culm, pendulous, the body 1.5 to 3.5cm. in length and 7 to 10mm. in width; perigynia broadly ovate, to rhombic-ovate, 3.5-4.5mm. by 2-2.5mm., with an oval scale ending in a 2mm. long awn. .... Subsp. *Maximowiczii*.

To the Eastern-Asiatic *C. Maximowiczii* are attributed two local variations, var. *suifunensis* originally reported from the easternmost part of Manchuria, and var. *levisaccus* from Japan. The latter is characterized by quite smooth perigynia and often thicker spikelets, but its area is perfectly included within that of *C. Maximo-*

wiczii s. str. Var. *suifunensis* with very cinereous smaller perigynia less than 3.5 mm long and fewer spikelets on more rigid culm is a northern Korean population, and is recently found at one locality in central Honshu of Japan as a quite local existence. As illustrated in Fig. 17, *C. Maximowiczii* s. str. covers all Japan and also extends as far as to central Korea, where it contacts with var. *suifunensis*, of which area extends northwards up to Suifun in eastern Manchuria. The southernmost record of *C. Maximowiczii* s. str. from Okinawa is based only upon a specimens collected there by Sakaguchi (KYO!), but since then no other person has found it in Okinawa nor it has ever been recorded in the islands lying between Okinawa and the southern part of the mainland of Kyushu, the datum is doubtful to some extent.

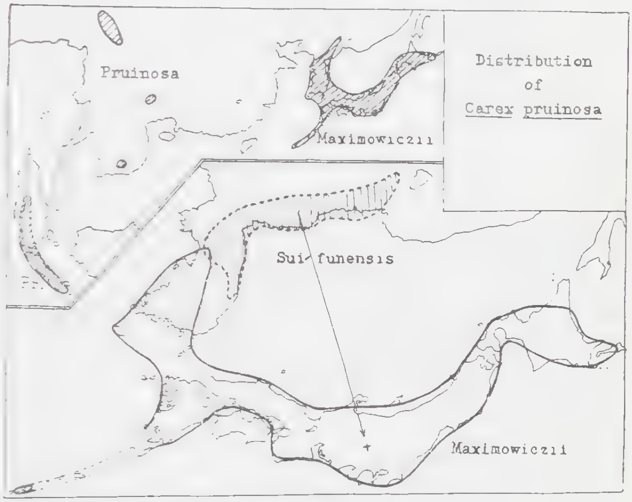
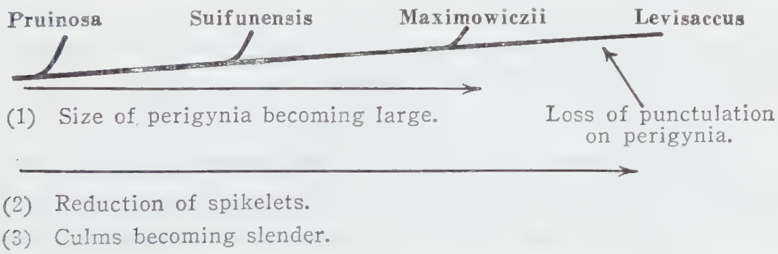


Fig. 22

The migration route of *C. Maximowiczii* into Japan is explicable by the Korean-Japanese Land Bridge. It has long been said that at least by the late Tertiary, the mainland of Japan is believed to be connected with the Korean Peninsula by a land bridge, through which many continental plants might have reached Japan. But on the other hand, the distribution of var. *suifunensis* gives us a clue to analize the process of speciation of *C. Maximowiczii* s. lat. Var. *suifunensis* was found at the foot of M. Yatsugatake in central Japan (cf. T. Koyama in Journ. Jap. Bot. **30**: 134. 1955), where we have noticed many northern Chinese plants, for instance *Crataegus pinnatifida* Bunge, *Geranium soboliferum* Komarov, etc. The group of plants is thought to have once occupied a more larger area in Japan in the Tertiary. Var. *suifunensis*, therefore, would have also spread in Japan at the time or so, and now its area must have restricted owing to the climatic factors, being followed by the differentiation of var. *Maximowiczii* s. str. Var. *levisaccus*, the smallest local popula-



tion, would have been born further from *C. Maximowiczii* s. str. It is interesting that some morphological characters in the following tendency also correlated well with this series of specific differentiation as given below.

(3) *Carex nubigera* D. Don—Fig. 23.

C. nubigera presents a typical distribution pattern of Himalayan-Japanese Elements. Its main area spreads widely over the highland of Himalaya from Afghanistan eastwards to Hupeh and Yunnan in China. The fragmental branch areas are scattered in montane regions of Ceylon and southern India, Sumatra and Java, Formosa, and in the lowlands in Japan. Two morphologically differentiated variants are found, *C. albata* of the northern Japan, and var. *Franchetiana* of the western Japan. In *C. nubigera* s. str. from Himalaya ovate-elliptical pale perigynia 4 to 5 mm. in length compose rather dense spikes with an elongate leaflike bract at the base. Japanese *C. albata* differs from typical *C. nubigera* chiefly in its larger habit, more dense inflorescence, brown-tinged scales, and deltoid-lanceolate perigynia terminated by a longer brownish beak, up to 5.5 mm. long. Var. *Franchetiana*, intermediate between the two, comes closer to *C. nubigera* than to *C. albata*, and differs from the former by its shorter perigynia 4 to 4.5 mm. long and spikes without any leaflike bract at the base. Fig. 23, bottom indicates the different processes of migration of the two into Japan. The area of *C. albata*, lying in northern Japan rather on the Japan Sea side, proves that the plant was distributed to Japan through the northern route, whereas the area of var. *Franchetiana* shows that it is the typical continental element, extended into Japan through the Korean-Japanese Land Bridge. Var. *Franchetiana* has once been wrongly treated as a variety under *C. albata*, but from this view both *C. Franchetiana* and *C. albata* should be coordinate infraspecific taxa attributed to the Indian *C. nubigera*.

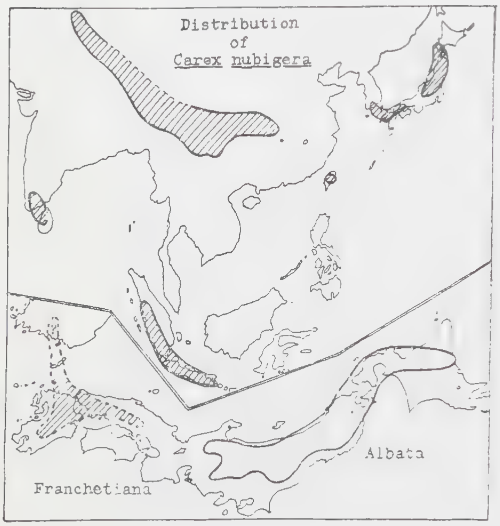


Fig. 23

Another similar case is observed in *C. Jackiana* Boott and its Japanese population, subsp. *parciflora* Kükenthal. As its taxonomy has already been discussed (T. Koyama in Bot. Mag. Tokyo **70**: 352-357. 1957), subsp. *parciflora* includes two large variants, var. *parciflora* and var. *macroglossa*. The former, a typical Boreal-Japanese Element, is interpreted as having migrated to Honshu from northwards, while the latter is thought to have entered into Honshu through Korea and afterwards ex-

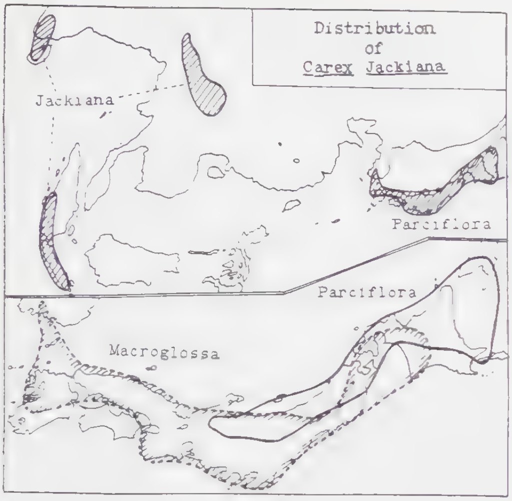


Fig. 24.

tended northwards to Hokkaido (Fig. 24). In *C. nubigera*, the morphological differences among its local populations are still very slight so that they are treated as varieties, however, the more advanced speciation seen in Japanese ssp. *parciflora* makes it possible to grant a subspecific status for it.

(4) *Carex ligulata* Nees ex Wight—Fig. 25.

In the phytogeographical respect, the occurrence of *C. ligulata* in Shikoku, western Japan, is the only difference from the above mentioned cases of *C. fedia* and *C. pruinosa*. Also a notable thing is that *C. ligulata* var. *austro-koreensis* is known as a variant in southern Korea. Since all the specimen from the mainland of Japan quite well agrees with those from India and China taxonomically, the Korean variety differing from typical one in more glaucous leaves and culms with more densely flowered spikelets and lighter colour of scales, is regarded to have been relatively newly differentiated from the typical *C. ligulata*.

Fig. 25. Distribution of *Carex ligula*.

Therefore, the migration route of this plant into Japan can be the so-called Korean-Japanese land bridge mentioned in the previous paragraphs. This route is just like one which the Japanese botanists have accommodated to a group of Chinese continental plants occurring in western outer Japan, called the Sohayaki elements. But the true Sohayaki elements are a group of relict species distributed in Kyushu, Shikoku, and Kii peninsula, their area sometimes further extending a little eastwards to western Tokai district, while the area of *C. ligulata* is larger, covering also northern Kyushu and Chugoku district of Honshu. Thus *C. ligulata* can not be treated as belonging to the Sohayaki elements.

C. Rochebruni, *C. teinogyna*, *C. maculata* and possibly *C. poculisquama* present the similar distribution pattern to that of *C. ligulata*. *C. Rochebruni* has a local variety in northern Formosa, named as var. *remotispicula*. The area of *C. maculata* is more extensive covering also northern Ryukyus, and its arm stretches out also to Java and the Moluccas. The Okinawan variety is named var. *Tetsuoi*. *C. teinogyna*, however, is not much differentiated. I agree with Nelmes in regarding the Japanese plants as quite identical with the Indian plants. *C. poculisquama* Kükenth. is a good ex-

ample of relict, known only from three localities, Nanking in China, Akiyoshidai in the westernmost part of Honshu, and Mt. Iwafune in central Honshu, Japan. This very rare sedge is expected to be found in the Himalayas.

(5) *Carex olivacea* Boott—Fig. 26.

C. confertiflora from Japan and *C. recurvisaccus* from Kwantung in China are the closest to *C. olivacea* of Himalaya and Java. Kükenthal and others are of opinion that *C. olivacea* and *C. confertiflora* are identical. Franchet and Ohwi, however, dis-

Table exhibiting the differences among *C. olivacea* aud its allies.

Part \ Taxon	*Olivacea*	*Recurvisaccus*	*Confertiflora*
Perigynia	broadly obovoid or ellipsoid-obovoid, 3-4.5 by 1.5-2 mm., beak about 1 mm long, recurved, conical, bidentulate	brordly ellipsoid or ellipsoid, 3.8-5 by 1.2-1.5 mm., beak conical-cylindrical, less than 1 mm long, obliquely truncate to bidentulate at criffice	broady obovoid to almost orbicular, 3.8-4 by 2-2.5 mm., beak bented down, cylindrical 1 mm long, bidentulate
Scale of pistillate flower	brown-red, rounded at muticous apex	brown-red, obtuse at muticous apex	pale, oblong, acute to acuminate at tip with an awn up to 3 mm. long
Spikelets	5 to 9 of which 1 to 2 being male, the body of female ones 3-16 by 0.5-0.8 cm., rather dense-flowered	5 to 7 of which 1 to 2 male, the body of female one 7 to 11 by 0.6-0.8 cm., rather loose-flowered	3 to 6 of which 1 (-2) male, the body of the female ones 2.5-5 by 0.7-0.9 cm., very dense-flowered
Leaf sheaths	brown-red	brownish to yellow-brown	brownish-pale

Fig. 26. Distribution of *Carex olivacea* s. lat.

tinguish these two sedges. Comparing the two authentic specimens of *C. olivacea** with *C. confertiflora*, I am of opinion to separate *C. confertiflora* as a subspecies of *C. olivacea*. *C. recurvisaccus*, in my opinion, differs from either of them, and the main distinguishing characters are given in the above table. The recent discovery of *C. recurvisaccus* from Kwantung, China, strongly suggests that these three are also related to each other phytogeographically, though the areas are so isolated. When the distribution illustrated in Fig. 26 is compared with that of *C. ligulata* in Fig. 21, it is natural to explain that they fall into the same category. The case of *C. olivacea* s. lat. must be a more advanced degree of speciation than in *C. ligulata* s. lat. Namely the large area in western China Proper must have pos-

* Himalaya, *Jenkins* (K)!, Sikkim, *Hooker f.* (K)!

sively gone out of existence.

Only from the data of distribution of Japanese ssp. *confertiflora* itself, it is impossible to consider the route by which this plant had migrated into Japan. But, the taxonomical link basing upon the morphological characters between *C. olivacea* and its spp. *confertiflora* gives a credit to regard ssp. *confertiflora* as it would have been extended into Japan directly from the central Chinese existence which is now much depleted with the retrogression of the area.

As fully discussed above, the three migration routes are observed as to the distribution of the Himalayan-Japanese species of Carices into Japan. The first route coincides with what the Japanese taxonomists named the Central-China-Japan direction to accommodate the so-called Sohayaki Elements. The Carices of this group suggesting this route, always occur in western Japan including southern Kyushu, Shikoku and the Chugoku District, but the reason why it is not adequate to regard them as the true Sohayaki Elements is that they have much larger area where they grow rather luxuriantly, thus seem to be probably younger and not to be relics.

The second route is the so-called Korean-Japanese Land Bridge. The sedges of this group which are thought to have migrated into Japan through this course, have never been noted in Shikoku. Usually their areas agree with those of the Manchurian and northern Chinese Elements distributed in the Chugoku District in Japan, including, for example, *Celtis Leveillei* Nakai, *Rhododendron mucronulatum* Turcz. etc.

The third is the northern route passing through Saghalien and Hokkaido southwards. The sedges belonging to this group are found in the northern parts of Honshu centering around the deep snow region just like the so-called Boreal-Japanese Elements.

C. ligulata Nees, *C. pruinosa* Boott, and *C. fedia* Nees are examples of the first, the second, and the third geographical category respectively here stated. Further, it is noteworthy that *C. nubigera* D. Don and *C. Jackiana* subsp. *parciflora* Kükenthal have two geographical local populations each, one belonging to the Boreal-Japanese Elements, and another being a kind of the northern Chinese and Korean Elements found in the Chugoku district of western Japan.

Nomenclatorial treatments

1. **Carex** (*Hirtae*) **fedia** Nees ex Wight, Contrib. Bot. of India 129 (1834); V. Krecz. in Komarov, Fl. URSS. **3**: 417 (1935)—*C. Wallichiana* Prescott (ex Wallich, List Pl. East Ind. Comp. Mus. 118. 1828, nomen tantum) ex Nees in Wight, Contrib. Bot. India 129 (1834); Kükenthal, Cyper.-Caric. 749 (1909)—non Sprengel (1826).

var. **fedia.**

var. **pilifera** (Kükenthal) T. Koyama, comb. nova—*C. Wallichiana* Nees var. *Miyabei* Kükenthal forma *glabrescens* Kükenthal, Cyper.-Caric. 749 (1909)—*C. drymophila*

Turcz. var. *pilifera* Kükenthal, Cyper.-Caric. 756 (1909)—*C. drymophila* Turcz. var. *udensis* Kükenthal, Cyper.-Caric. 755 (1909) pro parte, excl. basionym.—*C. glabrescens* (Kükenthal) Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. Ser. B, **6**: 245 (1931) excl. pl. e. Japon., et l. c. **11**: 509 (1936).

var. **Miyabei** (Franchet) T. Koyama, comb. nova—*C. Miyabei* Franchet in Bull. Soc. Philom. Paris 8^{e} sér., **7**: 52 (1895); Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. Ser. B, **11**: 508 (1936); Akiyama, Caric. Far East. Reg. As. 238, t. 243 (1955)—*C. saruensis* Franchet, l. c. 85 (1895)—*C. Wallichiana* Presc. ex Nees var. *Miyabei* (Franchet) Kükenthal ex Matsumura, Index Pl. Japon. **2** (1): 138 (1905); Kükenthal, Cyper.-Caric. 749 (1909). Japanese name: *Birôdo-suge.*

2. **Carex** (*Acutae*) **pruinosa** Boott in Proc. Linn. Soc. **1**: 225 (1845); Kükenthal, Cyper.-Caric. 352 (1909); Nelmes in Reinwardtia **1**: 428 (1951); T. Koyama in Le Natur. Canad. **82**: 204 (1955) et in Contrib. Inst. Bot. Univ. Montréal No. 70, 19 (1957).

subsp. **Maximowiczii** (Miquel) Kükenthal, Cyper.-Caric. 353 (1909); Nakai, Fl. Korean. **2**: 311 (1911)—*C. Maximowiczi* Miquel in Ann. Mus. Bot. Lugd.-Batav. **2**: 150 (1866); Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. Ser. B, **11**: 292 (1936); Akiyama Caric. Far East. Reg. As. 85, t. 55 (1955).

var. **Maximowiczii**—*C. Maximowiczii* Miq. var. *minor* Akiyama, Consp. Carlic. Japon. 100, f. 49 (1932), et Caric. Far East. Reg. As. 85-6 (1955). Japanese name: *Goso.*

var. **levisaccus** Makino et Nemoto, Fl. Japan ed. 2, 1446 (1931), sub *C. pruinosa*—*C. Maximowiczii* Miq. var. *levisaccus* (Makino et Nemoto) Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. Ser. B, **5**: 277 (1930) et l. c. **11**: 294 (1936); Akiyama, l. c. 86 (1955). Japanese name: *Hoshinashi-goso.*

var. **suifunensis** (Komarov) Kükenthal, Cyper.-Caric. 353 (1909)—*C. suifunensis* Komarov in Acta Horti Petrop. **18**: 445 (1901)—*C. Maximowiczii* Miq. var. *suifunensis* (Komar.) Nakai ex Kitagawa in Bot. Mag. Tokyo **48**: 373 (1934); T. Koyama in Journ. Jap. Bot. **30**: 134 (1955). Japanese nome: *Chosen-goso.*

3. **Carex** (*Multiflorae*) **nubigera** D. Don in Trans. Linn. Soc. **14**: 326 (1825); Kükenthal, Cyper.-Caric. 145 (1909); Nelmes in Reinwardtia **1**: 434 (1951); Ohwi et T. Koyama ex Kitamura in Kihara, Fauna & Fl. Nepal Himalaya 87 (1955).

subsp. **nubigera** var. **nubigera**—*C. fallax* Steudel, Synops. Pl. Glumac. 189 (1855)—*C. nubigera* D. Don var. *fallax* (Steud.) C. B. Clarke in Journ. Linn. Soc. **37**: 5 (1904)—*C pseudò-arenicola* Hayata, Ic. Pl. Formos. **6**: 118, f. 35, f-j (1816)—*C. fallax* Steudel var. *pseudo-arenicola* (Hayata) Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. Ser. B, **11**: 248 (1936).

var. **Franchetiana** Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. Ser. B, **5**: 283 (1930); Akiyama, Caric. Far East. Reg. As. 52, t. 19 (1955). Japanese name: *Tsukushi-minoborosuge.*

subsp. **albata** (Boott) T. Koyama, stat. novus—*C. albata* Boott (ex Miquel in Ann. Mus. Bot. Lugd.-Batav. **3**: 193, 1867, nomen; Franch. et Savat., Enum. Pl. Japon. **2**: 553, 1879, nom. seminud.) ex Franchet in Nouv. Archiv. Mus. Paris 3^{e} sér., **8**: 216 (1896); Ohwi, l. c. **11**: 246 (1936); Akiyama, Caric. Far. East. Reg. As. 52, t. 18 (1955)—*C. nubigera* D. Don var. *albata* (Boott) Kükenthal ex Matsumura, Index Pl. Japon. **2** (1): 123 (1905); Kükenth., Cyper.-Caric. 146 (1909). Japanese name: *Minoboro-suge.*

4. *Carex Jackiana* Boott. Nomenclature: T. Koyama in Bot. Mag. Tokyo **70**: 352-357 (1957).

5. *Carex ligulata* Nees. Nomenclature: T. Koyama in Acta Phytotax. et Geobot.

16: 40 (1955).

6. *Carex Rochebruni* Franch. et Savat. & var. *remotispicula* Ohwi. Nomenclature: Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. Ser. B, **11**: 257-8 (1936) and Akiyama, Caric. Far East. Reg. As. 66 (1955).

7. **Carex** (*Graciles*) **teinogyna** Boott, Illustr. Garex pt. 1, 60, t. 158 (1858); Kükenthal, Cyper.-Caric. 602 (1909); Nelmes in Mém. Mus. National Hist. Nat., N. S. Sér. B, **4** (2): 146 (1955)—*C. teinogyna* Boott var. *scabriculmis* Kükenthal, l. c. 602 (1909)—*C. scabriculmis* (Kükenth.) Ohwi in Acta Phytotax. et Geobot. **2**: 27 (1933). Japanese name: *Fusanakirisuge.*

8. **Carex** (*Anomalae*) **maculata** Boott in Trans. Linn. Soc. **20**: 128 (1846); Akiyama, Caric. Far East. Reg. As. 120, t. 105 (1955), incl. f. *viridans* Kükenthal.

var. **maculata.** Japanese name: *Tachi-suge.*

var. **Tetsuoi** (Ohwi) T. Koyama, stat. nov.—*C. Tetsuoi* Ohwi ex Ohwi & T. Koyama in Misc. Rep. National Sci. Mus. No. 5, 2, t. 2 (1952). Japanese name: *Ryukyu-tachisuge.*

9. **Carex** (*Molliculae*) **olivacea** Boott in Proc. Linn. Soc. **1**: 286 (1845); Kükenthal, Cyper.-Caric. 617 (1909, pro maxima parte!; Nelmes in Reinwardtia **1**: 395 (1951).

subsp. **confertiflora** (Boott) T. Koyama, stat. nov.—*C. confertiflora* Boott ex A. Gray, Bot. Japan 418 (1859); Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. Ser. B, **11**: 476 (1936); Akiyama, Caric. Far East. Reg. As. 148, t. 138 (1955)—*C. olivacea* Boott var. *angnstior* Kükenthal, Cyper.-Caric. 618 (1909). Japanese name: *Miyama-shirasuge.*

subsp. **recurvisaccus** (T. Koyama) T. Koyama, stat. nov.—*C. recurvisaccus* T. Koyama in Japan. Journ. Bot. **15** (2): 166, f. 2 (1956).

N. B. As to the name of the section to which *C. clivacea* belongs, I agree with Nelmes (Reinwardtia **1**: 395, 1951), who is of opinion that the section Tumidae Kükenthal is relatively heterogeneous but is difficult to separate into more than two groups. Being Tumidae Kükenthal a later homonym of Tumidae Meinsh., Molliculae Ohwi is nomenclatorically correct to accommodate to this section. A name Confertiflorae is the oldest, but in Franchet's opus, this name appears as a nomen nudum without any definite rank, and further, in its original sense, Franchet's Confertiflorae incidentally did not include neither *C. confertiflora* nor *C. olivacea.*

Sectio **Molliculae** Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. Ser. B, **11**: 450 (1936)—*Tumidae* Kükenthal, Cyper.-Caric. 611 (1909), non Meinshausen (1901)—*Confertiflorae* Franchet ex Ohwi, loc. cit. 474 (1936), non Franchet in Nouv. Archiv. Mus. **10**: 106 (1898), nomen. Species typica: *C. mollicula* Boott. Etiam pertinent *C. olivacea* Boott, *C. alliiformis* C. B. Clarke, *C. purpureotincta* Ohwi, *C. oedorahampha* Nelmes, *C. japonica* Thunb., *C. aphanolepis* Franchet & Savat., *C. planiculmis* Komar., *C. subtransversa* C. B. Clarke, *C. Brownii* Tuckerm., *C. transversa* Boott, *C. alopecuroides* D. Don, et *C. neo-Petelotti* Raymond.

摘　要

§22. ヒマラヤ・日本要素のスゲ類——いわゆるヒマラヤ・日本要素と考えられるスゲはビロードスゲ・ゴウソ・ミノボロスゲ・コジュズスゲ・サツマスゲ・ミヤマシラスゲ・ニコスゲ及びそれらの変種や亜種で

ある。この節ではこれらの分類学的な諸考察をまとめている。挿入した分布図が示す様に分布域の中心はヒマラヤ山地にあり，その腕が日本やスマトラ・ジャバに伸び出していて，それらの間に著るしい不連続が見られる。中国本部は特に広い干渉地域であつて，日本産の植物には本文でそれぞれ亜種や変種の扱いをした様な種々の程度の分化が見られる。この要素のスゲはマレーシアに分布する場合はスマトラ西部からジャバに連なる山地帯以外にあらわれないのも一特徴である。結論として得た関係事項は文末の学名変更によつてあらわされている。

Plasmolysis in Characeae

by Toshio HAYASHI* and Eiji KAMITSUBO**

林　俊郎*・上坪英治**： 車軸藻類の原形質分離

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Of various plant cells there are not a few materials in which plasmolysis does not take place readily. The internodal cell of Characeae is one of them. Strong adhesion of the protoplast to the cell wall may probably be one of the reasons which make plasmolysis difficult in this material. Yet it is not impossible to have them plasmolyse slowly using a suitable plasmolyticum.

As we recently had opportunities to observe various interesting and unusual phenomena in cells of *Chara Braunii* on the course of long-lasting plasmolysis in calcium nitrate solution, we should like to report them together with the behaviour of protoplast in some other plasmolytica in the following pages.

Material and Method

Internodal and "branchlet" (or "leaf") cells of *Chara Braunii* were used throughout the experiments. The plasmolyticum, which was mainly used for the present work, was calcium nitrate at the concentration of about 0.2 M. Besides, NaCl, $NaNO_3$, KCl, KNO_3, $CaCl_2$, $MgCl_2$, $MgSO_4$ and sucrose were also used for comparison.

The plant was cut off at the locus several cm. below the apical end. The freed portion consisting of several young internodal cells together with many "branchlet" cells was immersed in the plasmolyticum in a small Petri-dish. Observation was made mostly leaving the material in the Petri-dish. When a higher magnification was needed, the material was brought carefully onto a glass slide for microscopic observation.

All experiments were carried out at room temperature (10–15°) in autumn and winter. Renewal of the plasmolyticum was conducted with an interval of around a week.

Results

1. The plasmolysis with $Ca(NO_3)_2$ solution

The Course of Plasmolysis: Soon after the cell is immersed in the solution, a

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lot of small hollows are formed over the surface of the protoplast. These small hollows are incorporated with one another in a few minutes to form larger but fewer hollows, which are gradually transformed into sand-glass-like narrowings. The narrowed portions get more and more slender with time to form very thin strands which are eventually broken. The protoplast is then divided into two or more individual pieces exhibiting a convex-plasmolysis form. It takes 2–3 days for the protoplast to reach this stage after the treatment. Successive stages of deformation of the protoplast in the course of plasmolysis are shown diagrammatically in Fig. 1.

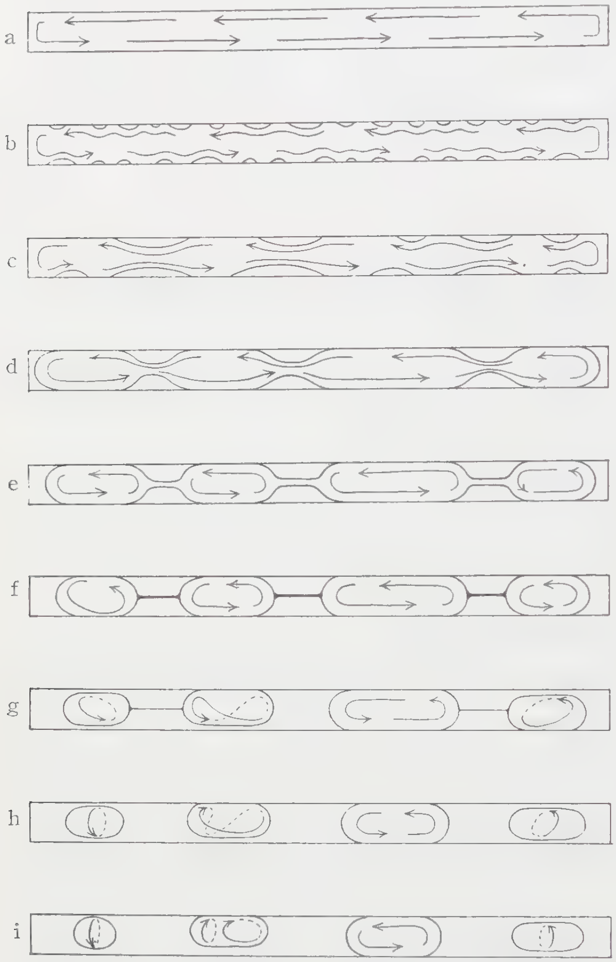

Fig. 1. Diagrams showing the course of plasmolysis in Characeae. *a*, before plasmolysis. *b*, soon after the cell is immersed in 0.2 M $Ca(NO_3)_2$. *c*, 1/2–1 hour later. *d*, about 24 hours later. *e*, the stage of sand-grass-like form. *f*, *g*, 2–3 days later. *h*, *i*, 10 or more days later. The arrows indicate the directions of the protoplasmic streaming.

Deformation and Behaviour of the Protoplast: About a week after the cell has been plasmolysed, the protoplast diminishes their volume spontaneously to a considerable extent. The decrease in volume of the protoplast is brought about not only by decrease in their length but by decrease in their width. Reduction in width amounts sometimes to one half of its initial width.

According as the volume of protoplast becomes smaller, the layer of the streaming endoplasm becomes thicker and a number of chloroplasts anchored in the cortical layer are freed from it. The freed chloroplasts are thrown into the streaming plasmasol, where they are carried along passively by the stream.

Although most of the protoplasts in a long internodal cell are dead after a week or so, the protoplasts found at the end of the cell, or protoplasts which are nearly spherical, survive often for two weeks, the protoplasmic streaming being kept

active during that time. On the other hand, in small cells of branchlets, the streaming continues very actively throughout several weeks, sometimes even more than 10 weeks under the plasmolysed state.

Protoplasmic Streaming: When the cell is brought into the solution, the protoplasmic streaming stops only for a few seconds before the cell resumes the streaming with an approximately normal rate in the same direction as before. Later, the speed of protoplasmic streaming gradually decreases and the streaming course, too, changes variously concomitantly with the deformation of the protoplast. In a week or two we are encountered with many startling and instructive patterns of protoplasmic streaming in the protoplast of the plasmolysed cell. Some of them are shown in Figs. 2-8.

As is well known, the streaming course of protoplasm in a "branchlet" cell is nearly parallel to its longitudinal axis. The course of streaming, however, is gradually altered after the cell has been thrown into plasmolysis. After 10 days the protoplasm often streams at right angle to the longitudinal axis of the cell ("right angle streaming").

Most probably the shifting of the streaming course is caused by the change in the orientation of the cortical layer which is supposed to be responsible for directing the streaming (Breckheimer-Beyrich[1]), Kamiya and Kuroda[2]), Hayashi[3])). The cortical plasmagel is gradually directed in right angle to the cell axis as the longitudinal contraction of the protoplast proceeds. In Fig. 1, f—i show some of the transitional forms leading to "right angle streaming".

The speed of streaming decreases gradually as the volume of the protoplast decreases, but it differs from protoplast to protoplast and also from part to part of one and the same protoplast (Fig. 3). Three weeks after plasmolysis, the speed becomes about 1/3 of that of the control. The decrease in speed goes hand in hand with the contraction of the protoplast.

Arrangement of the Chloroplasts: The regular arrangement of the chloroplasts is gradually disturbed as plasmolysis proceeds. In the early stage of plasmolysis, the orderly arrangement of chloroplasts is still maintained but the indifferent zones (white lines) become often obscure.

The arrangement of chloroplasts is modified little by little until it finally becomes disorderly. In some protoplasts, the streaming continues even when almost all chloroplasts are thrown out of place into the streaming plasmasol (Fig. 4).

As mentioned already, a number of chloroplasts become free from the cortical layer with the progress of plasmolysis and are carried along passively by the plasmasol involved in streaming. Sometimes, these chloroplasts form a large aggregate which is moved around with the streaming protoplasm while rotating around its own axis (Figs. 3, 5). We are also encountered not infrequently with a case in which the aggregate does not move and yet the plasmasol around the aggregate is still kept streaming (Fig. 6).

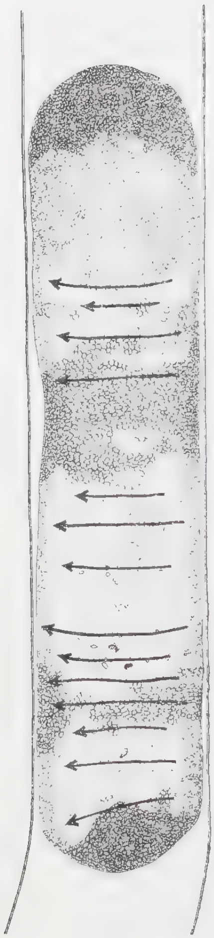

Fig. 2

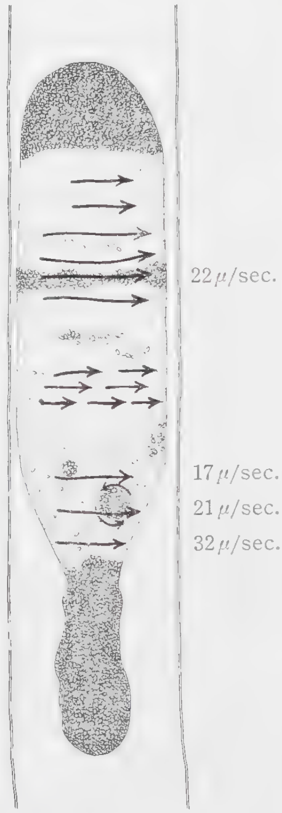

Fig. 3

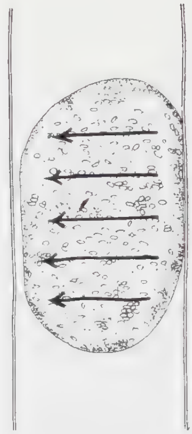

Fig. 4

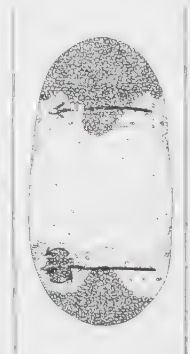

Fig. 5

Fig. 2. A protoplast of *Chara Braunii* ("branchlet" cell). 72 days after the treatment. The length: about 600 μ, the width: about 130 μ. Streaming rate: 24 μ/sec.

Fig. 3. The streaming rate differs from part to part in this protoplast. An aggregate of chloroplasts is moved around by the streaming protoplasm while rotating around its own axis. 22 days after the treatment. Length: 850 μ, width: 185 μ.

Fig. 4. A protoplast in which almost all chloroplasts are thrown out of place into streaming plasmasol. After 43 days.

Fig. 5. A protoplast with the aggregates of chloroplasts resting at the two ends. The plasmasol rotates around the longitudinal axis of the protoplast on each of these quiescent aggregates of chloroplasts. A free smaller aggregate of chloroplasts is rotating in the stream of the plasmasol. The middle space of protoplast is occupied by a vacuole. The cell sap is moved along by the streaming of protoplasm on both sides. 43 days after the treatment.

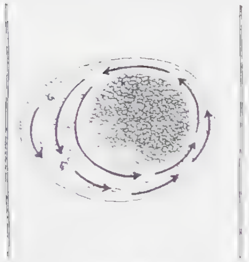

Fig. 6

Fig. 7

Fig. 8

Fig. 6. The stream of the plasmasol around a large central aggregate of chloroplasts which does not move and is attached to the peripheral layer of the protoplast. 74 days after the treatment. Streaming rate: 20 μ/sec.

Fig. 7. A protoplast having a small vacuole which is carried along slowly with the protoplasmic streaming. Streaming rate: 34 μ/sec. 11 days after the treatment.

Fig. 8. A protoplast having a doughnut-like vacuole. The central column of the protoplasm crossing the vacuole rotates around its own axis in the direction indicated by small arrows while other plasmasol streams in the opposite direction as shown by large arrows.

The Vacuole: The contraction of protoplast takes place at the expense of the decrease in the vacuolar space. When the cell is subjected to plasmolysis for several weeks, the vacuole becomes extremely small and is sometimes carried along slowly with the protoplasmic streaming (Fig. 7). Finally the vacuole disappears completely and a vacuole-free protoplast is obtained.

2. Behaviour of the cell in plasmolytica other than $Ca(NO_3)_2$

In order to compare the above behaviour of the cell in $Ca(NO_3)_2$ with that in several other plasmolytica, experiments were also conducted using NaCl, $NaNO_3$, KCl, KNO_3, $CaCl_2$, $MgCl_2$, $MgSO_4$ and sucrose. Concentrations of these plasmolytica, which are iso-osmotic to 0.2 M $Ca(NO_3)_2$, are about 0.27, 0.27, 0.27, 0.27, 0.19, 0.18, 0.44 and 0.46 M, respectively. But for the defference in plasmolyticum the method and the material were the same as before.

The Na- and K-salts are very toxic to this material at the concentrations used. In these solutions, the protoplasmic streaming is stopped just after the cell is immersed in them. An irregular concave-plasmolysis is observed for only about ten minutes before deplasmolysis starts. After 20–40 minutes complete deplasmolysis is accomplished accompanied with cell death.

In the case of Mg-salts, the toxicity is slight as compared with Na- or K- salts. In a few cases the protoplasmic streaming can be observed for three hours under

the plasmolysed state though the rate of flow is very small. Spontaneous deplasmolysis is not observed in the solution of Mg-salts, but the cell is killed several hours after it has been brought into the solution the contour of their plasmolysed forms being maintained unaltered.

There is no noticeable difference between $CaCl_2$ and $Ca(NO_3)_2$ so far as the plasmolytic behaviour of the cell is concerned, since various interesting phenomena described before are observed also in $CaCl_2$ solution several days after plasmolysis.

Sucrose is somewhat toxic as compared with Ca-salts. In this solution, many cells died in a few days, but some surviving cells exhibit a typical convex-plasmolysis as in the case of Ca-salts.

Considering the fact that the osmotic pressure of the cell in this material is equivalent to 0.22–0.24 M solution of sucrose, it is well to be expected that the 0.46 M sucrose contracts the protoplast into half the volume of the protoplast before plasmolysis. It is, however, to be noticed that in the solutions of Ca-salts, the volume of the plasmolysed protoplasts steadily decreases with time reaching only 1/4–1/8 of the original cell volume about 10 days after the treatment.

These experiments indicate that Na- and K-salts are fairly permeable to the cell of *Chara* and kill it soon after their application; the Mg-salts are not so permeable as to cause deplasmolysis, but they likewise kill the cell in several hours because of their toxicity. Ca-salts apparently hardly enter the cell; it is probably due to the very gradual leakage of monovalent, native ions from the cell under the state of plasmolysis that the protoplast never stops completely to contract in the solution of calcium salts. Sometimes very thin and incomplete membranes are observed to be left behind the contracting protoplast, which is well known in Siphonales tube and other materials.

Discussion

Plasmolysis is influenced by, among various factors, the degree of adhesion between the protoplast and the cell wall. In the case of *Chara* or *Nitella*, a gigantic size of the cell and the relatively delicate cell wall are also factors which make the plasmolysis difficult in these materials. Generally, when a long cell is plasmolysed, the protoplast tends to be divided into several smaller, independent portions. This tendency is also shared by the cell of Characeae.

Küster[4] who described in detail the behaviour of the protoplasmic streaming in plasmolysed *Elodea* cells, showed various abnormal and remarkable patterns of protoplasmic motion. The "belt-like streaming" observed by him probably corresponds to the "right-angle streaming" observed by us in *Chara*. A similar streaming pattern is also observable in *Nitella flexilis* and in root hairs of *Hydrocharis Morsus ranae* (unpublished). We thus see the general tendency that the streaming course takes the shortest pass when the cell is thrown into plasmolysis.

The reason why the streaming course is changed from the longest to the shortest under the plasmolysed state is an important problem which awaits further study.

At any rate it has been demonstrated in the present paper that the protoplasmic streaming is maintained for a remarkably long time, sometimes even more than 10 weeks, under the plasmolysed state.

A variety of streaming patterns in the plasmolysed protoplasts mentioned above are quite instructive in considering the mechanism of protoplasmic streaming, especially in locating the seat of motive force production. Further descriptions of the behaviour of the streaming and its analysis will be dealt with elsewhere by Kamitsubo.

Summary

Internodal and "branchlet" cells of Characeae can be plasmolysed by $Ca(NO_3)_2$ during as long as 1-10 weeks while keeping the protoplasmic streaming active. In the course of the long-lasting plasmolysis the protoplasts are subjected to a remarkable deformation, and concomitantly, the streaming pattern of protoplasm suffers pronounced modifications.

The authors wish to express their cordial thanks to Prof. N. Kamiya of Osaka University for his directions and encouragement throughout this work. One of the authors, Hayashi, wishes to express his gratitude for the financial aids from the Yukawa Fellowship of Osaka University.

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摘　　要

車軸藻類の細胞は一般に原形質分離をおこしがたい材料であるが，分離剤として Ca 塩をもちいることにより，比較的容易にこれをおこさせ，ながく分離の状態に保つことができる。約 0.2 M の $Ca(NO_3)_2$ 液中に材料 (*Chara Braunii* の節間および "葉" 細胞) をひたすと，はじめ原形質体表面に多数の小さいくぼみができ，それらは間もなく互に融合していくつかの大きいくぼみ (凹型原形質分離) となる。ひきつづき原形質体は 1～数個所で両側面からくびれて 2～数個の球状の分離原形質体となる (処理後 2～3 日)。その後これらの分離原形質体は，主として細胞の長軸方向に収縮をつづけながら，数週間—ときとしては 10 週間以上も生存をつづける。

よく知られているように，車軸藻類の細胞内にはさかんな原形質流動がみられるが，この流動は，原形質分離にともない流速・流動方向に種々の変化をきたす (例えば流速の減少や "横まわり")。

これらの現象は Ca 塩によつてのみ見られ，Na, K の塩類では数十分，Mg 塩では数時間で細胞が死ぬ。しよ糖を用いた場合，原形質分離はある程度まで進行するが，Ca 塩の場合にみられるような原形質体のいちじるしい収縮は起らず，かつ，大多数の細胞は数日間で死ぬ。

Studies on the Browning and Blackening of Plant Tissues

V. Chlorogenic Acid and its Isomers in the Leaves of *Viburnum* species*

by Hidemasa IMASEKI**

今関英雅** 植物組織の褐変現象について*
第五報 ガマズミ属植物の葉におけるクロロゲン酸およびその異性体

Received April 6, 1959

Introduction

Several researches relating to the mechanism of the browning reaction in green leaves kept in abnormal state such as injury, provisional high temperature have been reported[1),2),3),4),5)]. A general interpretation about the reaction is that some brown substance is formed by enzymatic or non-enzymatic oxidation of phenolic compounds which existed in green leaves. Hattori *et al.*[3),4)], Weurman and Swain[5)], and Uritani *et al.*[6)] indicated that in some plants, chlorogenic acid is the substance which is oxidized by phenolase and the oxidation product may polymerize forming a brown substance. The reaction for which dopa was responsible was also reported[2)].

The author was interested in a well-known fact that leaves of the plants belonging to genus *Viburnum* are liable in changing into brown when they are heated or pressed between papers for preparing dried specimen. The present paper deals with the determination of phenolic substances responsible for the browning and the relation between oxidase systems and the phenolic compounds in *Viburnum* leaves.

Experimental

Extraction: Fresh leaves were chopped and extracted with ten times their weight of boiling ethanol (80%) for 30 min. and the ethanol extract was concentrated under reduced pressure in a stream of coal gas. The aqueous residue was filtered through a pad of celite by suction to remove chlorophyll and the clear filtrate was added with an excess of 10% neutral lead acetate and adjusted to pH 7.0 with dilute ammonium hydroxide. The lead salt was treated with 5% acetic acid followed by filtration, and dilute ammonium hydroxide was added to make pH 8.5. The lead

* IV. Chlorogenic Acid in the Leaves of *Nicotiana Tabacum:* M. Shiroya, T. Shiroya, and S. Hattori, Physiol. Plantarum, 8: 594 (1955).

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salt was, after washing by centrifugation, decomposed with hydrogen sulfide in ethanol. After removing lead sulfide, the filtrate was concentrated and the concentrate was subjected to paper chromatography.

Identification of Phenolic Compounds: Identification of phenolic compounds was made by Rf value, color reaction on paper chromatograms, U-V. absorption curve and alkaline hydrolysis with eluates of phenolic bands[7]. Ethanolic ferric chloride (2%), Hoepfner's reagent[8] and ammonia vapor were used as reagents.

Crude Enzyme Preparation: About 100 g. of fresh leaves were chopped and immediately homogenized in a Waring blender with acetone cooled with dry ice. The resultant acetone pulp was repeatedly washed with acetone until a green color disappeared in the washings, and dried *in vacuo* over conc. sulfuric acid. For the measurement of phenolase activity, the acetone pulp was sieved and the powder of about 60 mesh was used to make 2% enzyme suspension.

Measurement of Phenolase Activity: The Warburg's manometric apparatus was used. The composition of the reaction mixture was as follows: 1 ml. of McIlvaine's buffer, 1 ml. of enzyme suspension, 1 ml. of 1/100 M substrate solution in the flask and 0.2 ml. of 20% KOH in the center well with a roll of filter paper soaked partially in the liquid. The substrate solution was tipped into the enzyme suspension from the side arm at zero time. In case of the experiments when inhibitor or amine was added, 0.3 ml. of 1/100 M inhibitor solution or 1 ml. of amine solution was added to the above reaction mixture. Final volume was 4.2 ml. The temperature of the water-bath was 30°.

Results and Discussion

a. Phenolic Compounds of *Viburnum* species

Phenolic compounds found to be present in the 12 species of *Viburnum* were tabulated in Table 1. Since Rf values of the phenolic compounds are remarkably affected by room temperature, the amount and the purity of sample to be spotted, they are not so essential. On the other hand, each relative Rf value to chlorogenic acid did not so vary with conditions, but gave almost constant value. Then the relative value (designated as Rc) was used for the identification of the compounds.

As seen in Table 1, the phenolic compounds contained in the 12 plant species are set in four groups. These four compounds gave with various reagents color reactions quite similar to chlorogenic acid (Table 2). U-V. fluorescence with and without ammonia vapor and reactions with Hoepfner's nitrite reagent suggest that the four compounds were isomers of chlorogenic acid. This was confirmed by their alkaline hydrolysis; eluates from every phenolic band were hydrolyzed by potassium hydroxide, and caffeic acid was detected in the ether-soluble fraction of the hydrolysate and quinic acid in the ether-insoluble fraction. Therefore, it is no doubt that the four compounds found in the present materials are all quinic esters of

Table 1. Phenolic Compounds in *Viburnum* species.

Material		*Viburnum* phenols I	II	III	IV	Authentic Chl.
V. dilatatum	Rf	0.88	0.74	—	0.64	0.75
	Rc	1.17	0.99	—	0.85	1.00
V. erosum var. *punctatum*	Rf	—	0.76	0.70	0.64	0.75
	Rc	—	1.01	0.93	0.87	1.00
V. Wrightii	Rf	0.87	0.81	0.76	0.71	0.82
	Rc	1.09	0.99	0.94	0.90	1.00
V. Sieboldii	Rf	0.90	0.75	—	0.66	0.76
	Rc	1.18	0.99	—	0.87	1.00
V. tomentosum	Rf	0.84	0.74	0.68	0.63	0.74
	Rc	1.14	1.00	0.92	0.85	1.00
V. phlebotrichum	Rf	0.86	0.72	0.65	0.59	0.72
	Rc	1.19	1.00	0.92	0.85	1.00
V. Sargenti	Rf	0.85	0.76	—	0.64	0.75
	Rc	1.14	1.01	—	0.85	1.00
V. furcatum	Rf	0.86	0.75	—	—	0.75
	Rc	1.15	1.00	—	—	1.00
V. Awabuckii	Rf	0.86	0.74	0.68	0.63	0.75
	Rc	1.14	0.99	0.91	0.84	1.00
V. japonicum	Rf	0.84	0.78	—	0.64	0.78
	Rc	1.12	1.00	—	0.90	1.00
V. Carlesii var. *bitchuense*	Rf	0.87	0.81	0.76	0.69	0.80
	Rc	1.09	1.01	0.94	0.86	1.00
V. branchyandrum	Rf	—	0.81	0.76	0.68	0.80
	Rc	—	1.01	0.94	0.85	1.00

Developing solvent: *n*-butanol-acetic acid-water (4:1:2)
Chl.: chlorogenic acid.

Table 2. Color Reactions of Phenolic Compounds in *Viburnum*.

	Viburnum phenols	Chlorogenic acid
$FeCl_3$	Green to gray	Green to gray
Hoepfner's reagent	Orange	Orange
NH_3 vapor	Yellow	Yellow
U-V. fluorescence	Whitish blue	Whitish blue
U-V. fluorescence under NH_3 vapor	Greenish blue	Greenish blue

caffeic acid.

Rc values of Spot II in various solvent systems were almost 1.00, consequently it is obvious that the Spot II is chlorogenic acid itself. U-V. absorption spectrum of every compound was quite the same with that of chlorogenic acid. Uritani and Miyano[9] compared U-V. spectra of the isomers with the spectrum of chlorogenic acid itself and found a close resemblance among these spectra. This also supports an idea that the four compounds are all isomers of chlorogenic acid.

The isomers hitherto reported are iso-[10], neo-[11] and pseudochlorogenic acids[9]. Among these isomers isochlorogenic acid is the only one, of which structure, i. e.

position of caffeic acid attached to quinic acid, has been suggested. Recently, Sondheimer[12] discovered another caffeic acid derivative (named "Band 510") which gave a yellow color with sodium hydroxide, a green color with ferric chloride and had Rf value 0.59 in *n*-butanol-acetic acid-water (4:1:5) while chlorogenic acid was 0.66 (Rc=0.90). The calculated Rc values from the reported data of iso- and neo-isomers are about 1.20 and 0.84, respectively. In the light of the fact described above, it is considered that Spot I and IV are iso- and neochlorogenic acids, respectively. Spot III, which is usually found to be in a small quantity, is not pseudochlorogenic acid, but corresponds to "Band 510" in its Rc value. Although the experiment to elucidate the structure of Spot III has not been carried out, the author has an idea that the substance may be one of geometrical isomers of chlorogenic acid because of the fact that the substance is contained with chlorogenic acid as a minor constituent and has a close Rc value.

The results of the present investigation were summarized in Table 3. It is interesting that most of the plants contained not only chlorogenic acid, but also its isomers. The distribution of chlorogenic acid has been examined by many investigators from the various points of view, but these were confined to chlorogenic acid itself except for a few instances. The author believes that there must be some interesting problems related to the biosynthesis of chlorogenic acid and its isomers.

Table 3. The Isomers of Chlorogenic Acid in *Viburnum* species.

	Isochl.	Chl.	Spot III	Neochl.
V. dilatatum	+	+	−	+
V. erosum var. *punctatum*	−	+	+	+
V. Wrightii	+	+	+	+
V. Sieboldii	+	+	−	+
V. tomentosum	+	+	+	+
V. phlebotrichum	+	+	+	+
V. Sargenti	+	+	−	+
V. furcatum	+	+	−	−
V. Awabuckii	+	+	+	+
V. japonicum	+	+	−	+
V. Carlesii var. *bitchuense*	+	+	+	+
V. brachyandrum	−	+	+	+

b. Phenolase Activity of *Viburnum* species

The browning reaction in the leaves of *Viburnum* did not take place when oxygen was not supplied, or the leaves were strongly heated. The green leaves, which were heated for 30 sec. on a water-bath of about 70°, rapidly turned dark brown with remarkable decrease of chlorogenic acid and its isomers. This suggests that the reaction in *Viburnum* plants is also caused by enzymatic oxidation of chlorogenic acid as shown previously by other workers. For this reason, oxidizability of

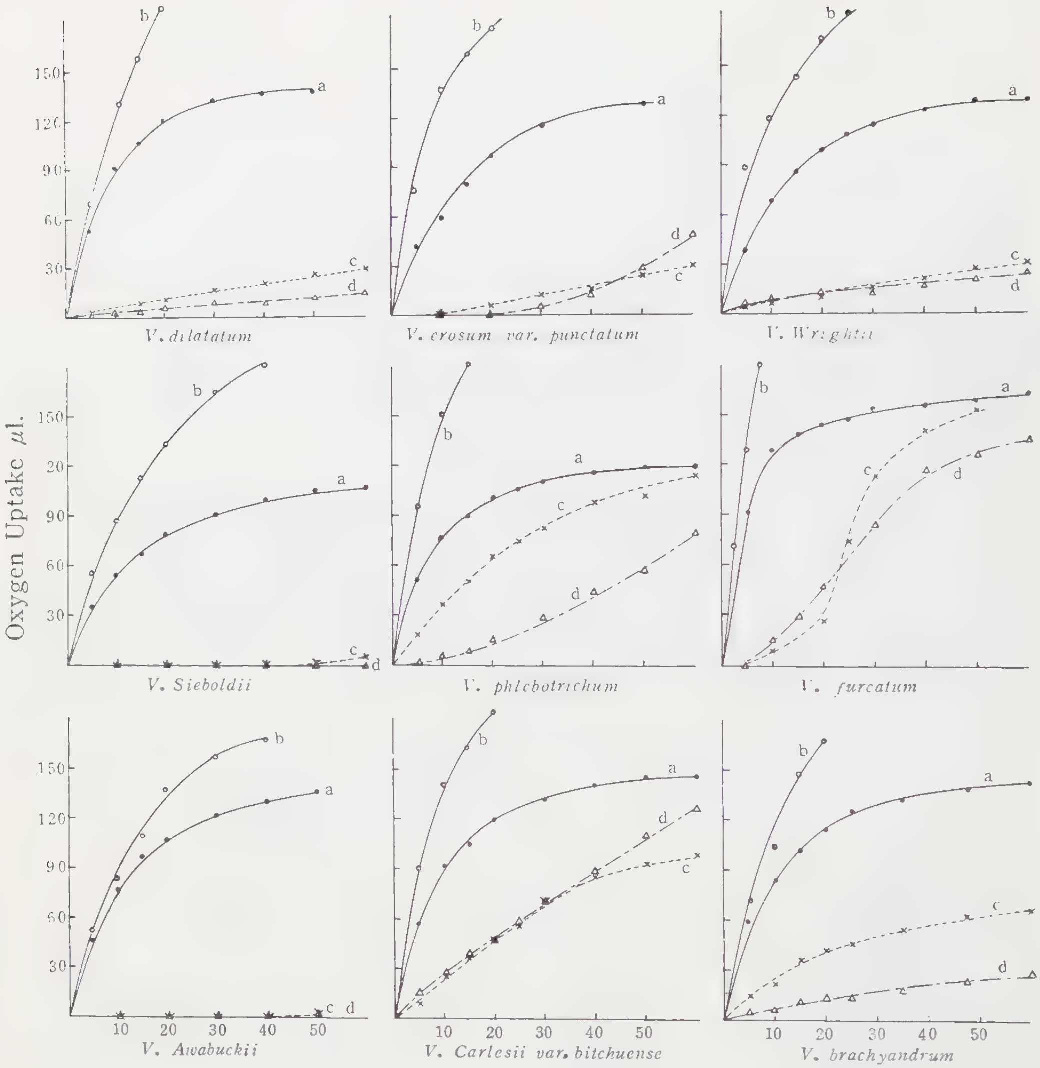

Fig. 1. Oxidation of chlorogenic acid by *Viburnum* phenolase in the presence of sulfanilic acid or inhibitors. Reaction mixture: 1 ml. of enzyme preparation, 1 ml. of 1/100 M chlorogenic acid, 1 ml. of McIlvaine's buffer (pH 6.8) and sulfanilic acid or inhibitors in the flask, 0.2 ml. of 20% KOH in the center well. Total volume: 4.2 ml. Curve a: none, b: 1 ml. of 5×10^{-2}M sulfanilic acid, c: 0.3 ml. of 1/100 M KCN, d: 0.3 ml. of 1/100 M diethyldithiocarbamate.

Viburnum plants for chlorogenic acid was examined by means of manometric method. The results (Fig. 1) show the presence of active oxidase activity. In course of the reaction, the color of the reaction mixture turned brown and the intensity of the color increased with the increase of oxygen uptake. However, relatively rapid inactivation took place as reported in the case of catechol-catecholase system[13].

The brown color formation and oxygen uptake were markedly inhibited by cyanide and diethyldithiocarbamate. On the other hand, the activity of phenolase

is promoted by addition of sulfanilic acid, and the rate of enzyme inactivation decreased. In that case, however, the pigment formed by oxidation of chlorogenic acid is not brown, but red. The red or purple color formation by enzymatic oxidation of catechol in the presence of primary amine such as aniline has been reported by Pugh *et al.*[14] and Jackson[15]. This phenomenon is interpreted as the aminoquinone formation by the reaction of amine and *o*-quinone which is produced by the primary step of catechol oxidation in the presence of catechol oxidase. As seen in Fig. 1, the addition of sulfanilic acid remarkably lowered the rate of enzyme inactivation. This is presumed to be a result that sulfanilic acid acted as a trapping agent for quinone which was produced by the oxidation of chlorogenic acid and inhibited the phenolase activity. Complete disappearance of chlorogenic acid in the browned leaves suggests the presence of a certain trapping agent such as sulfanilic acid or aniline. Amino acids are also assumed to be one of the agents. Jackson and Kendal[16] reported a similar action of amino acid like glycine, alanine, glutamic acid and arginine. When phenylalanine was added to the reaction mixture of chlorogenic acid and phenolase preparation from *V. Awabuckii* or *V. Sieboldii*, the increase of the rate of oxygen uptake was observed (Table 4). The degree of pig-

Table 4. Effect of Phenylalanine on *Viburnum* Phenolase

Oxygen uptake (μl) of chlorogenic acid by *Viburnum* phenolase with and without phenylalanine. Reaction mixture: enzyme preparation 1 ml., 1/100 M chlorogenic acid 1 ml., 9.8×10^{-3}M phenylalanine (PA) 0.5 ml., McIlvaine's buffer (pH 6.8) 1 ml., 20% KOH (0.2 ml.) was placed in the center well. Final volume 4.2 ml.

Time in min.		5	10	15	20	30	50
V. Awabuckii	no	23	37	47	54	66	75
	PA	33	57	73	89	109	130
V. Sieboldii	no	20	32	44	50	60	70
	PA	25	40	53	63	77	94

ment formation was also enhanced in the presence of the amino acid. Although such effect of amino acid is smaller than that of sulfanilic acid, it suggests a possibility of participation of cellular amino acids or proteins as trapping agents for the primary oxidation product of chlorogenic acid: the quinone will react with amino radicals in protein and thereafter complex polymerization will take place producing brown substances. This presumption is supported by the fact that although the brown pigment formed by autooxidation in the leaf extract, which contains no protein, is soluble in organic solvent, the pigment produced in the leaves by heat treatment is extractable neither with ethanol nor acetone. Chlorophyll may also take part in the browning reaction, because the browned leaves after heating contain remarkably less extractable chlorophyll than fresh leaves.

In order to examine the nature of each phenolase from *Viburnum* species, optimum pH range was determined, but no significant difference was observed (Fig. 2). In

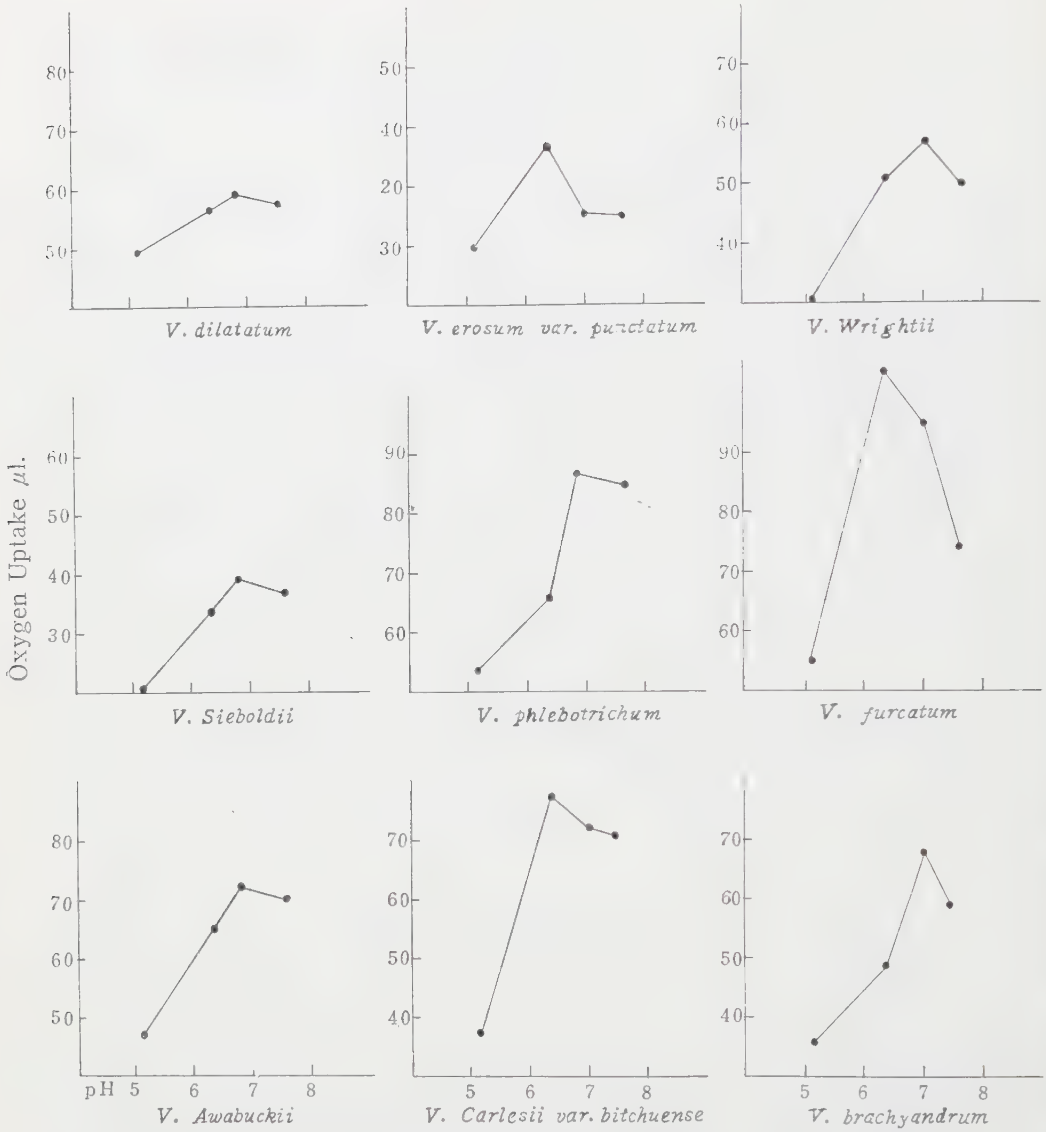

Fig. 2. Optimum pH of oxidation of chlorogenic acid by *Viburnum* phenolase. Oxygen uptake after 5 min. was measured in a reaction mixture of 1 ml. of enzyme preparation, 1 ml. of McIlvaine's buffer and 1 ml. of 1/100 M chlorogenic acid. 0.2 ml. of 20% KOH was placed in the center well. Total volume was 3.2 ml.

every preparation, the optimum pH existed in pH 6.5-7.0.

In the leaves of *V. furcatum*, there remains another possibility of the browning mechanism as proved in the leaves of *Aucuba japonica*[1]. The leaves of *V. furcatum* contain a glycoside furcatin, *p*-vinylphenol apiosylglucoside, and a glycosidase which hydrolyzes the glycoside into *p*-vinylphenol and apiosylglucose[17]. *p*-Vinylphenol is also oxidized by enzyme preparation from *V. furcatum* producing a light brown substance. Consequently, the reaction system that furcatin is first hydrolyzed and

then the aglycone is enzymatically oxidized giving a brown color may be possibly considered. However, semiquantitative estimation of furcatin on paper showed no significant difference in quantity of furcatin between fresh and browned leaves and components which markedly decreased were only chlorogenic acid and its isomers. It is, therefore, likely that the browning reaction system in *V. furcatum* related to furcatin is not essential.

Thus, the leaves of *Viburnum* species contain chlorogenic acid together with its isomers and oxidizing enzymes of the acids. Though the oxidizabilities of *Viburnum* phenolases on iso- and neochlorogenic acids were not examined, these acids are also assumed to be attacked by the phenolase from the points of view that phenolase is generally of wide specificity, and further these isomers as well as chlorogenic acid disappear during the browning reaction. Chemical estimation on the brown pigment formed in the leaves was not carried out, but the effect of some amine or amino acid on the browning reaction suggests the participation of proteins in the formation of brown substances.

Summary

1. Phenolic substances involved in the browning reaction in the leaves of *Viburnum* were examined. It was found that chlorogenic acid was the principal substance, which is always accompanied by the isomers.

2. A substance, which gives the same color reactions as chlorogenic acid but differs from the known isomers, was found in the seven species.

3. Phenolase activity was estimated in nine species and it was found that phenolase-chlorogenic acid system was responsible for the browning reaction.

4. The phenolase-chlorogenic acid reaction and the color formation were promoted by the addition of aromatic amine and amino acid, and a presumption as to the formation *in vivo* of brown substance was made.

The author wishes to express his cordial appreciation to Prof. S. Hattori and Ass. Prof. M. Shimokoriyama of the University of Tokyo for their kind guidance throughout the investigation. The author also appreciates to Dr. M. Hasegawa of the Government Forest Experiment Station, and to Mr. S. Nakamura and the members of the Nikko Botanical Garden for their kindness in supplying plant materials.

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摘　　　要

ガマズミ属の葉は傷害，温和な加熱などによつて，いちじるしい褐変をおこすことがしられている。本論文ではこの褐変に関係ある物質を同定し，その酸化系をしらべた結果を報告する。

1)　ガマズミ属における褐変の原因となる物質はクロロゲン酸およびその異性体と考えられる。 12 種の材料葉には例外なく，かなり多量のクロロゲン酸がふくまれ，同時にその異性体のどれかが，つねに存在する。

2)　クロロゲン酸と同一の呈色反応，組成をしめすが，既知のクロロゲン酸異性体（イソ，ネオ，プソイド異性体）とはちがつたフエノール成分が 7 種の葉に存在する。これはクロロゲン酸の幾何異性体の 1 つとかんがえられる。

3)　ガマズミ属の葉にはクロロゲン酸を酸化して褐色物質にかえる酸化系が存在する。この酸化系はシアン化カリウム，ジチオカルバミン酸ジエチルによつて阻害をうけ，最適 pH が大体 6.5～7.0 にある。また酸化反応は反応の進行にともなつて比較的はやい不活性化をしめす。

4)　酸化系はスルフアニル酸でいちじるしく反応が促進され，赤色の色素が形成される。フエニルアラニンでも同様の効果がみられた。

5)　したがつて，葉における褐変はクロロゲン酸の酸化，重合だけによるのではなく，ある種のアミン，アミノ酸，特に細胞タンパク質も褐変反応に関与しているとかんがえられる。すなわち，クロロゲン酸が酸化されてキノンとなり，これがタンパク質などのアミノ基と反応して褐色物質を形成するものと推定される。

ツユクサの青色メタロアントシアニンの結晶化とその性質とについて*（アントシアニンの研究，第31報**）

三井清司***，林　孝三***，服部静夫****

Seiji Mitsui***, Kôzô Hayashi*** and Shizuo Hattori****: Crystallization and Properties of Commelinin, a Blue Metallo-anthocyanin from *Commelina* (Studies on Anthocyanins, Part XXXI.)

1959 年 3 月 13 日受付

古来，花色変異の要因をめぐって提唱された主要な仮説として，われわれは Willstätter（1913）[illegible] pigment 説2)，柴田（1918）の金属錯塩説3) をあ[illegible]われてきたのは pH 説であるが，その後，柴田・林4)（1949）によって花色変異の問題が改めて検討された結果，その青色発現の機序は錯塩説によ[illegible]否を決するためには，天然の青色アントシアニンそのものの結晶化が不可欠であり，本研究はこの[illegible]

さきに著者5) らは野生のツユクサ *Commelina communis* L. var. *communis* の花から青色色素の結晶体を得て，その特性について報告したが，微量のため詳細な分析を行なうことができなかった。今回ツユクサの一[illegible]オバナ)***** *Commelina communis* L. var. *hortensis* Makino [illegible]によって青色色素成分の結晶化に成功した。この結晶体は，ツユクサから[illegible]認され，もっぱらこれを試料として定量分析を進めた結果，青色アントシアニン分子の構成がほぼ[illegible]になったので，一応この経過を報告することにした。

[illegible]については，目下研究の途上にあるが，今までのところ，アントシアニンによる青色の本体は，少くともツユクサでは，アントシアニン分子と金属元素とによって構成される有機金属[illegible]であることが示された。

われわれは，この新しい Metallo-anthocyanin を "Commelinin" と呼ぶことにする。

実　験　の　部

I．材　料

滋賀県草津市上笠町（旧　栗田郡山田村）で栽培されているオオボウシバナの花を用いた******。[illegible]後直ちに圧搾して得た濃青色の液から，下記の操作によって分離した青色色素[illegible]にして実験を進めた。

II．青色色素の単離および結晶化

[illegible] 7.03 kg. を手動圧搾器を用いて搾汁

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* 本報は著者らの 1 人（三井）の博士課程終了報告の骨子で，昭和34年 2 月21日に日本植物学会関東支部例会で講演した。なお要旨は Proc. Japan Acad. 35 (4) 169（1959）に発表した。

** 第30報：Proc. Japan Acad. 34 (6), 373（1958）

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***** この花の搾汁を和紙に塗って製した "青花紙" を材料として，さきに黒田チカ博士6) は無晶形の青色粉末を得て，それがカリウム，マグネシウム，アルミニウム……を含むという注目すべき知見を報告したが，[illegible]

****** [illegible]氏に，また材料の処理に便宜を与えられた三共株式会社　野州川工場長　梶村工博士ならびに社員の方々に厚く謝意を表する。

4.65 l. を得る。これに無水アルコール 25 l.（約 5.4 容)を加えてかきまわしたのち，しばらく放置し，青色物質の析出をまって吸引濾過する。濾液は淡黄色でわずかに青色を帯びるにすぎず，青色色素の沈澱はほとんど完全である。濾紙上の沈澱物は青色の無晶形粉末で，大量の夾雑物を含むが，同様光沢を呈する結晶性物質もわずかに含まれる。風乾量24g.。青色結晶体の精製にはアセトンによる分別沈殿も有効であるが，この場合はアルコールに比して結晶の収量が概して低下するようであるから，ここではもっぱらアルコールによる精製法について述べる。

精製 (1)——上記の粗物質24g. を冷水 160 ml. を用いて数回に分けてくりかえし抽出して，不溶の灰色繊維状物質を除去し，等量の無水アルコールを加えて氷室に 1 夜放置すると，ほとんど無色の無晶形物質を生ずる。これを除去した母液にさらに無水アルコール 160 ml. を加えて同様に不純物を沈澱させてのぞき，ついで無水アルコール 480 ml. を加えて全色素を沈澱させる。この操作によって不純物の大部分は除かれ，沈澱物の大部分は結晶性の青色粉末となり，母液は淡青色を呈するようになる。収量 14.5 g.。

精製 (2)——次にこの全量を冷水 150 ml. に溶解して濾過し，無水アルコール 150 ml. を加えて氷室に 1 夜貯えると，少量の無色無晶形の物質を析出するから，これを除いた濾液に，さらに無水アルコール 75 ml. を追加して氷室に放置すると，かなり大量の沈澱物が生成する。ほとんど全部が無色無晶形である。これを除いた母液にさらに無水アルコール 375 ml. を静かに加えると結晶性の青色物質が析出するから，そのまま氷室に 1 夜放置する。この際析出物のほとんど全部は結晶性となるが，なお少量の無晶形不純物が混ざっている。収量 10.2g.（風乾)。

精製 (3)——上記の析出物を水 75 ml. にとかして濾過する（不溶物はみとめられない)。濾液に無水アルコール 115 ml.（1.5 容）を静かに加えると，容易に青色稜柱状の針晶が析出しはじめる。この際不純物が析出するけはいがみとめられないから，さらに無水アルコール 35 ml.（0.5容）を器壁にそって静かに加え，そのまま氷室に 1 夜貯える。析出物はプリズム型針晶の集塊で，ほとんど純粋とみなしてよい。収量 5.2 g.（素焼板上に乾燥)。これを濾し集めたのちの母液に無水アルコール 75 ml.（合計 3 容のアルコールとなる）を加えて，室温（20°）で 1 夜放置すると，ほとんど同様の結晶 3.4g. が得られた。すなわち，粗結晶区分 10.2 g. からほとんど純粋な青色結晶体 8.6 g. が回収されたわけである。この母液にさらにに無水アルコールとエーテルを加えると，溶存する青色物質は完全に沈澱するが，これは少量であり，かつ純粋な結晶体にまで精製することはほとんど不可能である。

精製 (4)——以下の操作では再蒸溜によって精製した水およびアルコールを使用して上に得られた最初の結晶区分（5.2g.）について再結晶を行なった。青色結晶 5.2g. を冷水 50 ml. に溶かし，ゆるく吸引しつつ濾過するとなおわずかながら，淡灰緑色の不純物が濾紙上に残る。濾液に無水アルコール 100 ml.（2 容）を静かに加えて前回と同様にして氷室中で析出させると，顕微鏡下でまったく単一にみえるプリズム型針晶の集塊が得られる。収量 2.8g.。母液にさらに無水アルコール 50 ml.（計 3 容）を加えると，同様の結晶が析出する。収量 0.9g.。

この母液* からアルコールの添加によってさらに結晶 0.5g. が回収されるが，この区分は多少不純物をともなっている。

精製 (5)——上記の結晶区分は顕微鏡下の観察では，まったく単一であるが，完全に精製するため，さらに次のように粉末セルロース柱による操作を試みた。

上記の結晶区分 2.8g. を水 25 ml. に溶解し，この深青色の溶液を東洋濾紙粉末（100～200 Mesh）を充塡した柱（2×8cm.）に注ぎ，次いで 50 %アルコールを自然に流下させて，青色色素を十分に溶出して濃青色の流下区分を捕集し，無灰濾紙を用いてこれを濾過する(85 ml.)。この際濾紙粉末柱にはほとんど残留物が認められないが，ただその最上層がわずかに灰緑色を帯び，痕跡程度の不純物が夾在していたことがわかった。流下液 85

* この青色母液の一部をとって，酢酸マグネシウム飽和の含水アルコール溶液数滴を加えてしばらく放置すると，青色色素は結晶または無晶形に析出し，上澄液は完全に脱色する。したがって精製の際の物質の損失は，無機元素が部分的に失われることによると解される。

ml. について上述のように，無水アルコール添加によって 2g. の純物質を回収した。この物質は顕微鏡下で美麗な青色稜柱状の板状または針状の結晶で (Fig. 1)，乾燥粉末は美しい銅光沢を呈し，次のような諸性を示す。

III. コンメリニンの性質

コンメリニンの青色結晶標品は， 230° の近くでわずかに収縮するが， 290° 以下では融解しない。

その分析値は Tab. 1 のとおりである。

Fig. 1 Blue crystals of commelinin (×450)

Table 1. Elementary Analysis of Commelinin

Water of crystallization

Subst. (air-dried) :	Loss in wt. (115°, P_2O_5, 1 mm. Hg)	Found H_2O
1) 3.378 mg.	0.355 mg.	10.27 %
2) 2.829 mg.	0.275 mg.	9.72 %

C-H-estimation

Subst. (anhyd.) :	CO_2	H_2O	ash ;	C %	H %	ash %
1) 3.023 mg.	5.732	1.161	0.213	51.74	4.30	6.89
2) 2.554 mg.	4.808	0.989	0.223	51.37	4.33	8.73

N-estimation

Subst. (anhyd.)			
Subst. (anhyd.)	6.843 mg. :	N_2 0.0392 ml. (9°, 750 mm. Hg) :	Found N 0.693 %
〃	8.146 mg. :	N_2 0.0362 ml. (11°, 761 mm. Hg) :	Found N 0.537 %

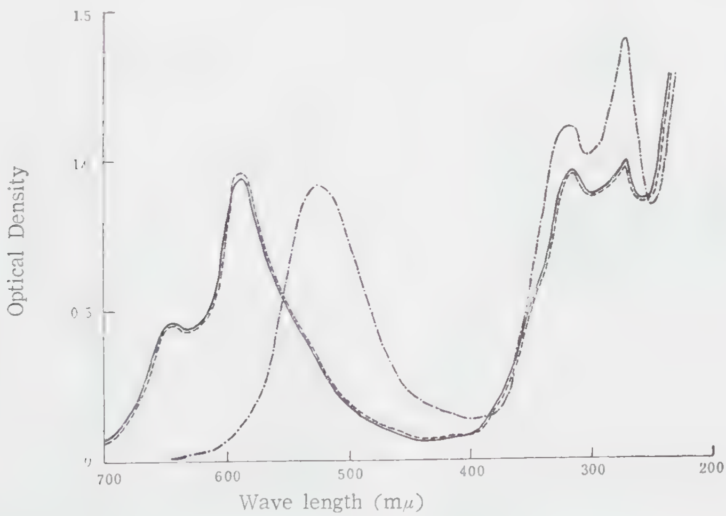

Fig. 2 Absorption spectra :

———— Commelinin (from var. *communis* and var. *hortensis*) in H_2O (4.43 mg. %) ; λ_{max}=643, 591, 316, 273 mμ.

- - - - - - 1 % -HCl-treated commelinin in H_2O (4.43 mg. %) ; λ_{max}=643, 591, 317, 273 mμ.

—·—·—·— Commelinin (4.43 mg. %) in 2 % HCl aq. (red solution) ; λ_{max}=528, 317, 274 mμ.

Dumas-Pregl 法によって N_2 として測定された上記のガス量は，Nを含まぬ試料を燃焼した場合にも同程度に認められたから，上記のN値は無視できるものと思われる。

コンメリニンを 1/40 モル・酢酸マグネシウム溶液（酢酸で pH 6.0 に調整）中で濾紙電気泳動を行なうと，250 V.，0.4 mA/cm.，4 hr. で(+)極方向に 23 mm. だけ泳動する。しかもその間に色素は完全に原色を保ち，分解の徴候はみられない。しかし，通常の赤色アントシアニンは同一条件下でわずかながら（—）極の方向へ泳動する。またコンメリニンは通常のアントシアニンと異なり，これを流水透析または電気透析しても，半透膜を通過しない。

さらに，この色素の水溶液について吸収を測定すると，λ max＝643，591，317，273 mμ（Fig. 2参照）で対応する赤色アントシアニンの場合とは著るしく異なる吸収特性を示す。

なお，コンメリニン水溶液は中性で，アルカリによって直ちに緑色に変るが，酸性側ではかなり広範囲の pH 変化に対して極めて安定に原色を保つ。またイオン交換樹脂または EDTA（Ethylenediamine-tetraacetic acid）によっても青色はまったく不変である。

以上の諸性質についてツユクサおよびオオボウシバナよりの結晶標品は完全に一致しており，さらに赤外線吸収においても，まったくちがいがみとめられない（Fig. 5 参照）。

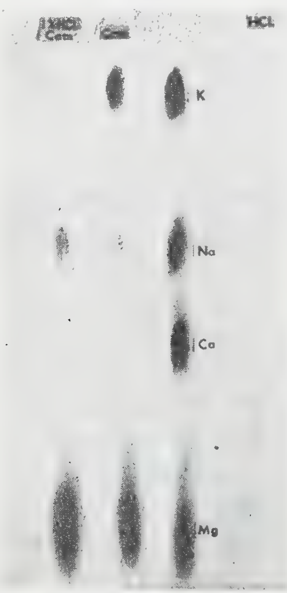

Fig. 3 Paper chromatogram (after Tristram) of metalic components of commelinin and HCl (1 %)-treated commelinin. (Irrigated with 94 %-EtOH on Tôyô No. 52 filter paper, descending, 23°, 18 hrs.)
HCl : 0.5 % HCl used as control. (Ash was dissolved in this solvent throughout.)
Com : ash (as chlorides) from commelinin.
1 % HCl-Com : ash (as chlorides) from HCl (1 %)-treated commelinin.

Ⅳ. コンメリニンの定性分析

(1) 有機成分：青色の Commelinin を 20 % HCl 中で3分間煮沸し，エーテル層，イソアミルアルコール層，水層の3層にそれぞれ分離抽出し，各部について定性試験を試みた。エーテル層からは *p*-クマール酸が融点ならびに濾紙クロマト法によって検出，同定され，イソアミルアルコール層では，濾紙クロマトグラフィおよび吸収曲線によって，アグリコンとしてデルフィニジン（塩化物）をまた水層からは同じく濾紙クロマトグラフィによってグルコースをそれぞれ確認することができた。このことからコンメリニンのアントシアニン成分はアオバニン（Awobanin)*（デルフィニジン—3：5—ジモノグルコサイド+*p*クマール酸）であることが知られる。

(2) 無機成分：コンメリニンの結晶を灰化すると，純白な灰分約7％が得られる。これはかなり吸湿性で一部は潮解する。希塩酸には発泡しながら溶解し，炭酸塩の混在が暗示される。この溶液について濾紙クロマトグラフィによって，金属元素の定性を試みた。

まず，*n*-ブタノール/酢酸/水（4：1：5）およびアセトン/*n*-ブタノール/塩酸（10：4：2）を展開溶剤とし，クサントゲン酸カリウム，フェロシアンカリウム，ルベアニン酸およびオキシン（8-ヒドロキシキノリン）を検出試薬として，ニッケル，コバルト，モリブデン，鉄，銅，アルミニウム，クロム，亜鉛などの重金属元素の検出を試みたが，すべて陰性であった。

* 黒田チカ博士[7]（1936）はオオボウシバナの搾汁から製した青花紙を用いて，そのアントシアニン成分をアオバニンと命名して組成を明らかにした。

しかし，灰分試料はジピクリルアミンの点滴反応において[illegible]ことから[illegible]は[illegible]，[illegible]としてアンモニアガスの上で紫外線で観察すると，黄緑色の螢光を発するスポットがみとめられた。これはマグネシウムであることが co-chromatography によって確められた。

そこで，Tristram[8] 法によって灰分中のアルカリ金属およびアルカリ土類金属を[illegible]したところ，[illegible]カリウム，マグネシウム，ナトリウムを検出することができた（Fig. 3 参照）。

さらに灰分の一部を酢酸に溶解して Erlenmeyer[9] 法に従って展開発色したクロマトグラムにおいても，[illegible]カリウム，マグネシウム，ナトリウム[illegible]。

V. コンメリニンの[illegible]

(1) 有機成分： コンメリニン中の *p*-クマール酸は無水の試料を水素気流中で 2*n*-NaOH に溶解し，室温下に約30分放置して鹸化した後，HCl-酸性としてエーテルで抽出し，脱水後重量法により定量した。実験値は無水試料（152.10 mg.）の 11.83%（17.99 mg.）であった。

次に，コンメリニンの無水物を 20%-HCl 中で 3[illegible]，[illegible]，アグリコンおよび糖をそれぞれエーテル，イソアミルアルコール，水[illegible]し，イソアミルアルコール層については Peckman 分光光度計による比色法（555 mμ）で，デルフィニジン（塩化物）を，また水層については T T C (Triphenyltetrazolium chloride)[10] 法にしたがってグルコースを比色定量した。

物質（無水）：	デルフィニジン（塩化物）	グルコース
① 9.582 mg.	2.613 mg. (27.27%)	2.839 mg. (29.63%)
② 21.105 mg.	5.781 mg. (27.39%)	6.201 mg. (29.38%)

なお，グルコースについては，別に無水物 84.54 mg. を水解分配後，Pertrand 法で糖を定量した際，27.00 mg.，すなわち無水試料の31.94%に相当するグルコース値が得られた。

以上の実験結果を平均するとコンメリニンに含まれる各有機成分の定量値は次のようになる。

p-クマール酸	11.83 %
デルフィニジン（塩化物）	27.33
グルコース	30.78
合　　計	69.94 %

(2) 無機成分： コンメリニン無水物を Pregl 法によって硫酸塩として灰化すると白色灰分6.27 [illegible]。この灰分を炎光分光分析* にかけて[illegible]したところ，次のような実験値**が得られた。ただし定量の基準とした輝線は，K : 768mμ，Na : 589mμ，Mg : 383，371mμ である。

コンメリニン（無水物）：	Mg(%)	K(%)	Na(%)
12.615 mg.	0.39	1.63	0.28
23.960 mg.	0.45	1.30	0.30
平　　均	0.42	1.47	0.29

VI. コンメリニンの1% HCl 処理について　かりに Willstätter の pH 説を肯定すれば，コンメリニンは酸処理によって容易にそのアルカリ金属を脱離して色調も青から赤へ変わることが予想される。

そこで，コンメリニン 500 mg. を 1% HCl 10 ml. に溶解し，室温で30分間放置してみたが，溶液の青色には何の変化もみられなかった。この[illegible]無水アルコール 50 ml. を加えると，直ちに[illegible]形の青色沈[illegible]が生成し，上澄は淡青紫色を呈する。[illegible]物を[illegible]しあつめ，85%アルコールで洗って乾燥する。収量 370 mg.

これを 10 ml. の水に溶解して濾過後，無水アルコール 20 ml. を静かに加えて2液層を形成させ，そのまま冷所に1夜放置すると，碧青色の板状結晶が析出する。収量 40 mg.

濃青色の母液にさらに 20 ml. の無水アルコールを静かに追加し，冷所に1夜放置して，針状ないし板状の碧青色の結晶 105 mg. を得た（Fig. 4 参照）。

融点はコンメリニンの原標品と同じく，290° 以下では融解しない。

この結晶標品をコンメリニンの原標品とならべて，50%（v/v）アルコールで上昇展開した濾紙クロマトグラムでは，両者とも Rf 0.77 を示し

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* 三共株式会社製剤研究室の妹尾節哉氏実施

** コンメリニンを Pregl 法で灰化して得られた無機元素の硫酸塩を希塩酸に溶かして炎光分析にかけた。なおコンメリニンの水溶液を直ちに炎光分析しても同様な分析値の得られることが判明した。

Fig. 4 Blue crystals of 1 % HCl-treated commelinin (×2000)

て，まったく一致した。また 1/40 モル・酢酸マグネシウム (pH 6.0) 中での濾紙電気泳動においても両者の泳動速度はまったく同一であった。赤外吸収スペクトル (Fig. 5) および可視部～紫外部の吸収スペクトル (Fig. 2 参照) に関しても，酸処理の前後において，まったく差異が認められなかった。

塩酸処理標品の元素分析の結果は Tab. 2 の通りである。

すなわち，塩酸処理を経た結晶標品では灰分（酸化物として）がかなり減少していることがわかる。

そこで塩酸処理コンメリニンの灰分（酸化物）を希塩酸にとかして濾紙クロマトグラフィを行なった。Tristram 法によって得られたクロマトグラム(Fig. 3 参照)は明らかにカリウムの消失を示しているがマグネシウムは希塩酸処理によっても失われないことが明らかに示された。なお，この際検出されたナトリウムはおそらく再結操作の途中で混入したものである*。

次に塩酸—処理コンメリニン（結晶）を Pregl

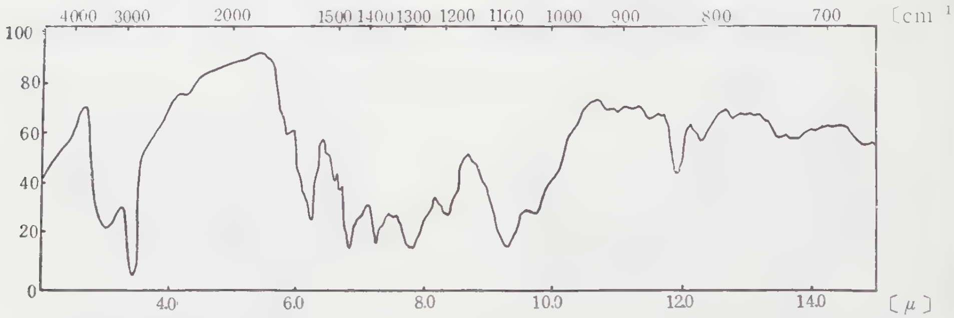

Fig. 5 Infra-red absorption spectrum of commelinin (from var. *communis* and var. *hortensis*) and HCl (1 %)-treated commelinin

Table 2. Elementary analysis of commelinin treated with 1 % HCl

Water of crystallization

Subst. (air-dried) :	Loss in wt. (115°, P_2O_5, 1 mm. Hg)	Found H_2O
1) 3.350 mg.	0.346 mg.	10.32 %
2) 3.077 mg.	0.298 mg.	9.69 %

C-H-estimation

Subst. (anhyd.) :	CO_2	H_2O	ash ;	C %	H %	ash %
1) 3.004 mg.	5.904	1.089	0.185	53.63	4.06	6.16
2) 2.779 mg.	5.449	1.060	0.123	53.51	4.27	4.43

N-estimation

Subst. (anhyd.)			
5.075 mg. :	N_2 0.014ml. (26°, 758 mm.Hg) :	Found N	0.13 %
〃 3.530 mg. :	N_2 0 :	〃 N	0

S-and Cl-estimation negative

* コンメリニンの精製途上の 2，3 の結晶区分については，ナトリウムがまったく検出されないことが見られたからである。

法により硫酸塩として灰化したところ，4.31%に[illegible]各金属元素の定量結果は次のとおりである。

物質（無水物）	Mg(%)	K(%)	Na(%)
① 9.445 mg.	0.44	0.06	0.51
② 18.605 mg.	0.55	0.02	0.40
平　均	0.50	0.04	0.45

また有機成分についても，前記の方法によって定量した結果は次のとおりであり，

物　質 [illegible]	デルフィニジン [illegible]	グルコース
① 9.050 mg.	2.580 mg. (28.51%)	2.769 mg. (30.60%)
② 9.858 mg.	2.730 mg. (27.69%)	2.899 mg. (29.42%)

コンメリニン[illegible]

他方コンメリニンの水溶液をイオン交換樹脂アンバーライト[illegible]流下液は濃青色でこれを自然蒸発するとき，原標[illegible]は，Mg が判然と確認できる。

[illegible]酸処理に際しても，青色が失なわれないかぎりは，マグネシウムもまた消失しないという事実は，後[illegible]示すものであろう。

考　　察

コンメリニン[illegible]によって，その分子組成の70%あまりが明らかに[illegible]される。これについては，たとえば，コンメリニンをそのまま濾紙に点着して酢酸/濃塩酸/水（5：1：5）で上昇法で展開する時，[illegible]に青色色素から分解生成した[illegible]色のアオバニンに相当するスポットのほかにフロントの近くにごく淡い黄褐色のスポットがみられることである。しかも，このものはアンモニアガスの上で紫外光下に，極めて鈍い黄褐色の螢光を発することが認められる。同様なことはn-ブタノール/酢酸/水(4：1：5）で上昇展開した場合にも現れる。しかし，[illegible]質に相当するものは紫外光下でも検出されない。

これらの事実は十分に再結精製した結晶標品で[illegible]

この淡黄褐色の物質を，濾紙クロマト法により分離したのち，温水で溶離して精製を試みたが，きわめて吸湿性にとむ水によく溶ける粉末として[illegible]シウム－塩酸の還元反応は陰性で，かつフェーリング試薬ならびに TTC の還元反応は陽性である。樟脳にはとけずアセチル化も不成功に終った。しかし，アルカリ熔融をすると，フェノール性分解[illegible]紙クロマトグラフィによって確認された。またフ[illegible]で，おそらくフラボノイドに近縁な物質であろう[illegible]270mμ にそれぞれ吸収極大が認められ，コンメ[illegible]いる。

[illegible]ら，コンメリニンはアオバニン，カリウム，マグネシウム（ナトリウム）とともに，この未知のフラボノイド様物質がその構成にあずかっているも[illegible]

[illegible]問題に対する積極的な解答はまだ得られないが，少なくとも pH 説の普遍性を実験的に否定するこ[illegible]は，柴田の錯塩説にその根拠を求めなければなら[illegible]

しかし，一方，未知のフラボノイド様物質の存在が認められるのであるから，Robinson の Co-

pigment 説もまた無視することはできないが，著者らは一応この未知物質は，アオバニンとマグネシウムの青色錯塩体に二義的に弱く結合することによってコンメリニンにおける青色の安定性に寄与していると考えたい。

このような観点から，かりな高分子と考えられるコンメリニン分子の組成を，これまでの定量結果から推定してみた (Table 3，4参照)。

Table 3. Analysis of Individual Components of Commelinin Molecule

Component	Molecular or atomic weight	Ratio of components	Theoretical value (as M. W.＝4900)	Experimental value
Mg	24.3	1	0.49%	0.42%
K	39.1	2	1.59	1.47
(Na)	23.0	1	0.47	0.29
Delphinidin (as chloride)	339	4	27.67	27.33
Glucose	180	10	36.73	30.78
p-Coumaric acid	164	4	13.38	11.83
Unknown subst.	300＋18 ?	4 ?	25.95 ?	......
Total			106.28	72.12＋ ?

Table 4. component analysis of 1%HCl-treated Commelinin

Component	Molecular or atomic weight	Ratio of components	Theoretical value (as M. W.＝4800)	Experimental value
Mg	24.3	1	0.50%	0.50%
K	39.1	0	0	0.04
(Na)	23.0	1	0.47	0.45
Delphinidin (as chloride)	339	4	28.25	28.10
Glucose	180	10	37.50	30.01
p-Coumaric acid	164	4	13.66	......
Unknown subst.	300＋18 ?	4 ?	26.50 ?	......
Total			106.88	......

以上の実験的事実から推定できることは，1原子のマグネシウムを中心としてアオバニン4分子がこれに配位し，さらに未知物質（おそらくは配糖体として）がこれに弱く結合しており，なおアオバニンのフェノール性水酸基のどれかが，カリウム(時にはナトリウム)塩となっているところの，分子量 約 4900 のメタロアントシアニンがコンメリニンであろうと想像される。

最近，E. Bayer[11] (1958) はヤグルマギク (*Centaurea cyanus* L.) の青色花から同様な方法で分離した青色色素の無晶形粉末について，無機元素の存在を証明して錯塩説の見解を支持したが，その際検出された主要な金属は鉄とアルミニウムである点が，コンメリニンの場合と異なっている。しかし，このことは今後なお青色色素の結晶化を待って再検討しなければならない。

著者らはこのような事実をも考慮しつつ，さらに研究を進めることによって，錯塩学説を詳細に

検討するつもりである。

終りに臨み，終始激励と有益な助言を賜わった [illegible]

する。なお材料の処理と試料の分析とに便宜を与えられた三共株式会社高峯研究所の松居部長，中村 [illegible]

Summary

(1) A blue anthocyanin was isolated in crystalline state from the petals of "Awobana"-plant (*Commelina communis* L. var. *hortensis* Makino) (cf. Fig. 1), and was shown to be identical in all respects with the blue crystals previously obtained from the petals of wild commelina (*Commelina communis* L. var. *communis*). We propose the name "commelinin" for this blue anthocyanin.

(2) Commelinin seems to be a high molecular compound, which is non-dialysable through semi-permeable membranes. The estimation of the individual components gave the following results: *p*-coumaric acid 11.83 %, delphinidin (as chloride) 27.33 % and glucose 30.78 %, Mg 0.42 %, K 1.47 % and Na 0.29 %, respectively. Probably, Na is not inherent to the pigment molecule.

(3) On treatment with 1 % hydrochloric acid, commelinin loses its alkali metals altogether. The resultant product, which is still bound with Mg as before, is also convertible into brilliant blue crystals (cf. Fig. 4). Therefore, it is concluded that the blue color of commelinin is not due to an alkali phenolate of anthocyanin.

(4) Mg remains fixed to the pigment molecule even after treatment either with EDTA or cation exchangers. Of course, no perceptible color change occurs in these cases.

(5) Besides, commelinin seems to contain an appreciable amount (25—30 %) of an unknown substance, which is pale yellowish in color and is presumed to be a flavonoid.

(6) To sum up the analytical results obtained, commelinin is a co-ordination compound, in which one atom of Mg combines four molecules of awobanin (delphinidin-3:5-dimonoglucoside + *p*-coumaric acid) around it, and an unknown flavonoid-like substance is further brought into association with it (cf. Table 3 and 4).

文　　献

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抄録

植物生長ホルモンのキレート結合

A. Cohen, D., Ginzburg, B. Z., and Heitner-Wirguin, C., Metal-chelating properties of plant growth substances. Nature : 686**181**—687 (1958).

B. Recaldin, D.A. and Heath, O.V.S., Chelation or complex-formation by indoleacetic acid *in vitro*. Ibid. **182** : 539—540 (1958).

最近キレート物質がオーキシンと同様に燕麦子葉鞘の伸長促進作用をもっていることがわかり(例えば Bennet-Clark, 1956 ; Heath and Clark, 1956), オーキシンも結局そのキレート作用によって伸長を促進すると考えられてきた。このことから，オーキシンが実際に金属とキレート結合するかどうかが試験管内で試みられることになった。

A : IAA と Cu $(NO_3)_2$ を用い，IAA-Cu 又は NAA-Cu の結合体ができることを absorption spectra と酸に対する滴定曲線 (Bjerrum 法) をしらべて証明した。そしてこの関係は Fe^{+++} と Co などの重金属においてのみなりたち，Ca や Mg にはなりたゝぬことを示し，オーキシンは carrier として重金属を mask 或は remove して重金属による細胞膜の生長阻害を除くと結論した。

B : これに対して Recaldin and Heath は同様の実験を行ない，Cu については同じ結論を得た。しかし Fe^{+++} に対しては異なった結論に達した。すなわち，IAA+Fe^{+++} の発色 (450～600mμ) や O_2 吸収，CO_2 発生及び Salkowski 反応をみて，IAA は Fe^{+++} の添加で2時間以内にその大部分が酸化的に分解されることをみて，IAA は Fe^{+++} とは結合しないことを知った。Cu では IAA は4.5時間後でも10%しか分解しないことが判った。

以上の論文をみると，IAA は Cu などの重金属とは高濃度の場合キレート結合をするが，Ca の移動が関係しているといわれる第1次伸長促進における IAA 細胞膜の loosening の機構はこれでは全く説明できない。 (増田芳雄)

ブルタニュー海岸の海藻の温度および滲透抵抗

Biebl, R., Temperatur-und osmotische Resistenz von Meeresalgen der bretonischen Küste. Protoplasma **50** : 217—242 (1959)

著者は1937年以来，おもに海藻の温度，光，乾燥，滲透の各抵抗性について研究してきた。この実験は1957年の夏にロスコフ臨海研究所で行なったものである。ブルターニュ海岸の50種類の緑藻，褐藻，紅藻について，低温 (−8, −2, 3°) と高温 (27, 30, 35°) の抵抗性と，稀釈および濃縮海水に対する滲透抵抗をしらべた。方法は海藻を各温度の海水中で12時間処理したあと，滲透抵抗の方は種々な濃度の海水に24時間処理したあと，その細胞の生死から抵抗性の限界をきめた。採集場所によって，(1)潮間帯のもの (2)潮だまり，低潮線のもの (3)それより深い所に生育する漸深帯 (5 −25m.) のものと，3つの生態的グループに分類した。温度抵抗は，3グループ間で明らかに異なり，特に低温の抵抗性に大きな差があった。漸深帯の海藻は，海水の凍結しない −2～−3° に生存の限界がある。0° 附近で死ぬものが多いが，潮間帯のものは−8°の低温にも耐えることができる。高温にたいしては大体30° まで生存できる。*Enteromorpha compressa* は特に強く，35°に12時間おかれても死ぬことはなかった。当海岸の海藻は，著者が前に研究した（1939）ナポリの漸深帯の海藻と温度抵抗範囲が似ている。漸深帯の紅藻の中には，極端に高温に弱い北方性のものと，寒冷に弱い南方性のものとの両方があることが確かめられた。滲透抵抗について，ロスコフの海藻はプリマス，ヘリゴランド，ナポリの海岸のものとよく一致しており，抵抗性と生育場所とが相関している。滲透抵抗範囲は潮間帯のもので0.1—3.0海水濃度（10～300%正常海水濃度），漸深帯の紅藻で 0.4—1.8 海水濃度であった。同じ深さに生育する海藻間で緑藻は褐藻，紅藻にくらべて特に滲透抵抗が大きいことがあげられている。

(照本 勲)

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第24回大会講演要旨を大会不参加の会員に一部送料とも 200 円で配布します。ご希望の方は仙台市片平丁東北大学理学部生物学教室内日本植物学会大会準備委員会あてご送金下さい。

Effect of ATP on the Rate of the Protoplasmic Streaming in *Nitella*

by Tadashi SANDAN* and Takao SOMURA*

山段　忠*・宗村隆生*：フラスモの原形質流動速度におよぼす ATP の影響

Received February 27, 1959

Goldacre and Lorch (1950)[1] demonstrated that the flow of endoplasm in *Amoeba discoides* occurred in distal direction from the site of 1-3% ATP injection. Kamiya *et al.* (1957)[2] reported that ATP (2×10^{-3}M) admitted from without markedly increased the motive force of the protoplasmic streaming in the plasmodium of *Physarum polycephalum* and that this effect manifested itself several minutes after the reagent was added and continued several ten minutes after the reagent was removed. Furthermore, using adenylic acid (AMP) from both muscle and yeast, Kamiya *et al.* could not observe any effect whatsoever comparable to that of ATP. Hatano and Takeuchi (1959)[3] showed the existence of ATP in the plasmodium of *Physarum polycephalum* at the concentration of 0.4×10^{-3}M. These experiments favor the assumption that ATP may serve as the direct energy source for the protoplasmic streaming just as it is for muscle contraction and other energy requiring physiological processes.

The effect of ATP on the protoplasmic streaming in plant cells which are equipped with cell wall, or dermatoplasts, has been studied so far but little except that on the protoplasmic motion in the stalk cell of *Acetabularia* (Takata, 1958)[4]. It is the purpose of the present experiment to investigate the effect of ATP upon the velocity of the protoplasmic rotation in the cell of Characeae.

Material and Method

From *Nitella flexilis*, the third internodal cell (ca. 2.5 cm. × ca. 400 μ) from the apex was isolated by means of a small scissors and used as the material. Thus isolated cells were kept in distilled water for more than 15 hours prior to the experiment so as to let the cells recover from harm possibly induced by the cutting of their adjacent cells. The experiments were carried out using a glass vessel as shown in Fig. 1. An internodal cell to be investigated was mounted in a definite medium in the concavity (C) which was subsequently sealed with a cover-glass (G). The temperature of the concavity was maintained nearly constant by a continuous flow

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of tap water (22.6 ± 0.05°C.) through the tubing (T) enveloping the concavity. The whole vessel was set on the stage of a microscope, and the rate of protoplasmic streaming was determined as the rate of flow of small particles in protoplasm by means of an ocular micrometer and a stopwatch. At first, the rate of protoplasmic flow in a cell placed in a Sörensen's phosphate buffer solution (pH 6.6, M/100) was estimated. This control measurement was continued for 20 minutes. Then, the medium was replaced with an ATP solution which was made by dissolving a certain amount of ATP (Na-salt)* into the buffer solution. After the effect of ATP was investigated for 70 minutes, the medium was again replaced with the plain buffer solution and the reversibility of the rate of streaming was observed. The effect of AMP (yeast adenylic acid)** at various concentrations on the protoplasmic streaming in the *Nitella* cell was also examined in a way similar to that mentioned above.

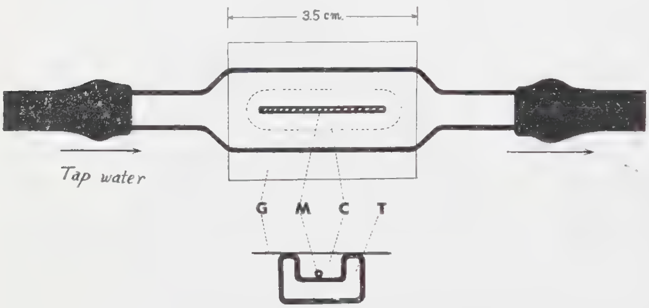

Fig. 1. Glass vessel for the determination of the rate of protoplasmic streaming at constant temperature.
C: concavity, G: glass cover,
M: material, T: tubing for tap water flow.

Results and Discussion

Changes in rate of the protoplasmic streaming caused by the addition of ATP at various concentrations are shown in Figs. 2–6. In these figures, the ordinates represent the rate of flow in microns per second, and the abscissas time in minutes; the time of the medium replacement is indicated with arrows.

As seen in these figures, the rate of flow was kept almost constant in the cells placed in the plain phosphate buffer solution and a temporary decrease in the rate of the streaming or even a momentary stoppage of streaming resulted from the addition of ATP. These changes in the rate of the streaming may be partly due to the possible mechanical shock caused by the replacement of the medium.

In the case of 5×10^{-4}M ATP (Fig. 3), the rate of flow dropped to 6/7 of the control as soon as the ATP solution was substituted for the plain buffer solution. In about 15 minutes, however, the flow did recover from this temporary retardation and further speeded up for a while, the acceleration being 6% of the control. This acceleration lasted about 40 minutes before the rate came back again to the control level.

* ATP (Na-salt) manufactured by 'Zellstofffabrik Waldhof', Germany.

** AMP manufactured by the Tokyo Chemical Industry Co., Ltd.

The maximum stimulating effect of ATP was found at 1×10^{-3}M (Fig. 4); the rate of streaming was promoted by 15% of the control. This acceleration was manifested with a time-lag of about 15 minutes and the accelerated level was maintained unchanged for more than 50 minutes. As ATP was removed, the rate of flow dropped to the initial control level within 10 minutes.

ATP at concentrations below 1×10^{-4}M could not affect the rate of flow. On the other hand, 1×10^{-2}M ATP slowed down gradually the streaming until it completely stopped within 70 minutes. The flow did not recover from this complete suspension even when ATP was removed. The streaming was completely and permanently stopped as soon as ATP at above 1×10^{-2}M was applied.

AMP ($<1\times10^{-3}$M) was found to cause little effect on the protoplasmic streaming. In Figs. 7 and 8 is illustrated effect of AMP added at the concentrations of 5×10^{-4}M and 1×10^{-3}M, the concentrations at which ATP accelerated the streaming. By addition of AMP ($>1\times10^{-3}$M), the streaming was always retarded.

Recently, Takata (1958)[4)]

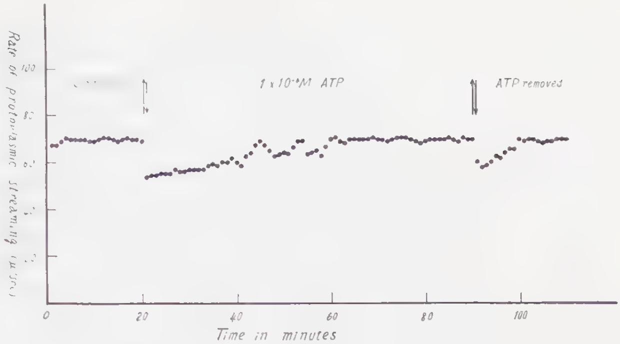

Fig. 2.

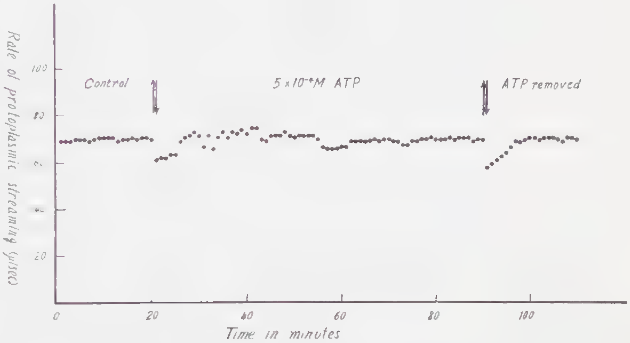

Fig. 3.

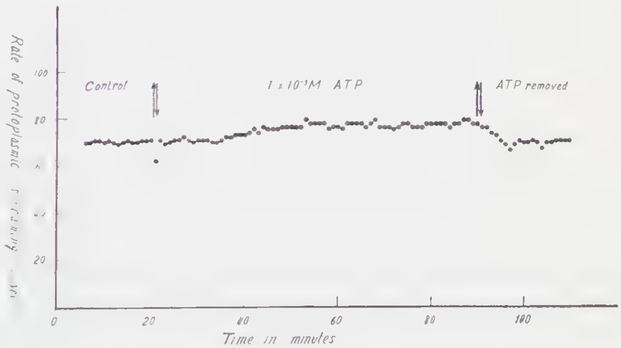

Fig. 4.

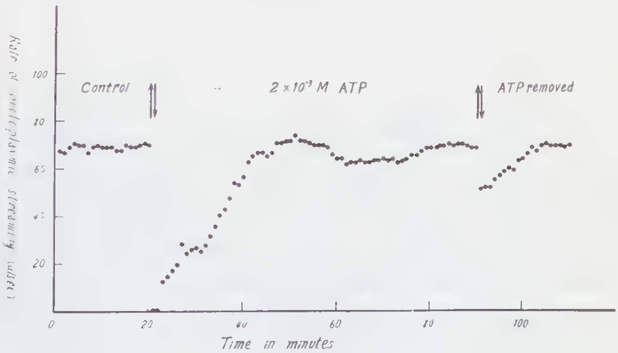

Fig. 5.

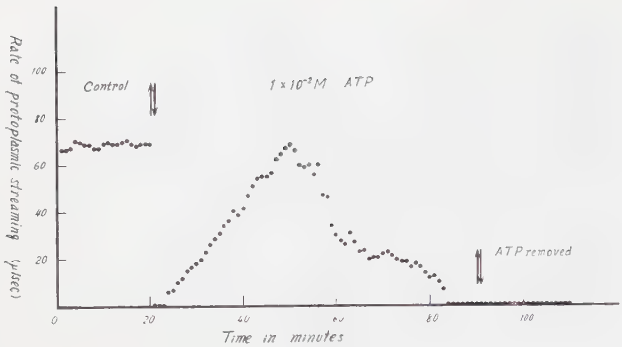

Fig. 6.

Figs. 2–6. The effect of ATP on the rate of protoplasmic streaming.

observed that 5×10^{-4}M ATP strongly increased the velocity of the protoplasmic streaming in the stalk cell of *Acetabularia calyculus.* But this promotion lasted only for a brief period of time. In the present work, the rate of protoplasmic rotation in the internodal cell of *Nitella* was found to be definitely accelerated, if the acceleration is not conspicuous in amount,

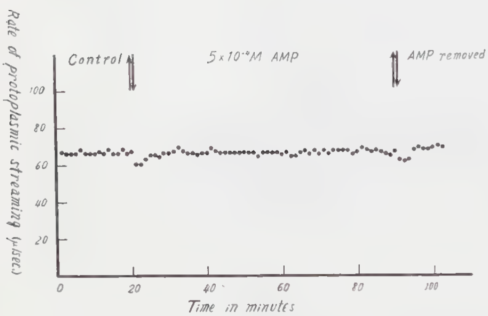

Fig. 7.

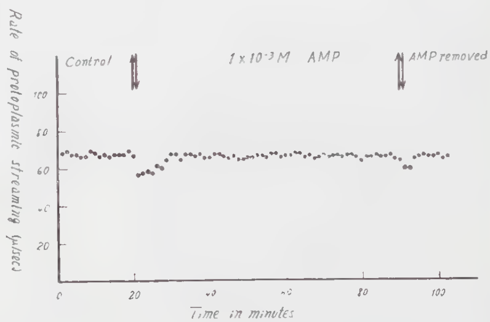

Fig. 8.

Figs. 7–8. The effect of AMP on the rate of protoplasmic streaming.

by the application of ATP at appropriate concentrations. Especially, 1×10^{-3}M ATP increased the velocity of streaming by 15% for more than 50 minutes. AMP gave no discernible effect of acceleration on the rate of the flow. The results of Takata's and the present experiments appear to suggest a high possibility that ATP may also play a major role in the mechanism of the protoplasmic motion in the cells of green plants.

Summary

The protoplasmic streaming in the internodal cell of *Nitella flexilis* was found to be slightly accelerated by the addition of ATP in adequate concentrations. Thus, in the most remarkable case, 1×10^{-3}M ATP increased the rate of the streaming by 15% with a time-lag of 15 minutes. The accelerated rate was maintained for more than 50 minutes. With the removal of ATP, the rate went down within 10 minutes to the initial low level. AMP gave no discernible effect of acceleration on the rate of streaming in the cell.

The authors wish to express their most cordial thanks to Prof. N. Kamiya of Osaka University for his kind direction and helpful criticism throughout this work.

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摘　　要

[illegible]節間細胞の原形質流動速度は適当な濃度のATP処理によって促進される。もっともいちじるしい効果は 1×10^{-3} M ATP でみられ，この場合流動速度は標準値の15% 程度高められる。その促進効果はATP 添加後約 15 分であらわれ，50 分以上続くが，ATP 除去によって 10 分以内に失われる。

AMP に関しては，いかなる濃度でも ATP でみられるようなあきらかな促進効果はみとめられない。

Studies on the Activities of Fermentation During the Growth of Bacteria*

by Akiko KASAMAKI,** Shoji SASAKI** and Shoichiro USAMI**

笠巻明子**・佐々木昭治**・宇佐美正一郎**： 細菌の生長過程における醱酵能について*

Received April 2, 1959

Many studies on the relation between the cultural age of microorganisms and their metabolic patterns or activities have been reported. It has been demonstrated that the protein and nucleic acid biosyntheses are most strong in the lag phase[1] and that the highest activity of deaminase is attained in the late lag phase or stationary phase[2]. Beside this, the relationship of the age of the cells to the adaptability of enzyme formation and to the resistance of cells against some antibial drugs has been the subject of many studies.

The previous work by the present authors revealed that the deaminase activity of *Proteus vulgaris* was influenced by the growth temperature and cultural age[3]. Subsequently, further studies were performed on the activities of several oxidases at the initial stage of growth; it was found that the enzymic activities in the log phase were generally higher than in the lag phase[4].

In this paper, the results of the studies on the changes in fermentative activity of bacterial cells during the course of growth are reported.

Method

Proteus vulgaris was used as the test organism.

Inoculum cell suspensions were obtained from cultures grown on usual pepton-broth agar slant for 18 hrs. at 30°. After washing, they were diluted with sterilized water to a definite turbidity in the photometer readings, all possible precautions being taken to avoid contamination.

Bacteria were grown by shaking culture in 1 per cent pepton-broth medium at 37° for each following time; 10, 20, 30, 40, 50, 60, 90, 120, 180, 240 and 300 minutes. At the end of the culture periods indicated, the medium was immediately cooled with ice, and then bacterial cells were harvested and washed twice with deionized water by centrifugation. They were then suspended in deionized water or Krebs-

* This work was partially aided by the Grant of Scientific Research from Ministry of Education. A part of it was delivered at the 22nd Annual Meeting of the Botanical Society of Japan, October, 1957.

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Ringer-Bicarbonate solution (for measurement of fermentation). These resting-cell suspensions were used for the experiments.

$Q_{CO_2}^{N_2}$ value was measured by the common method using CO_2-Krebs-Ringer-Bicarbonate buffer. As CO_2 output continued constantly as far as it was estimated, $Q_{CO_2}^{N_2}$ values were calculated from the volume of CO_2 for the first 60 minutes. The conditions of the measurement were as follows: M/500 $NaHCO_3$ was used with a gas mixture consisting of N_2 and CO_2 in a ratio of 9 to 1. In this case, partial pressure of CO_2 was calculated from the equation of Henderson-Hasselbach. A small quantity of O_2 in this gas was removed by means of yellow phosphorus. After suction, the vessel of a Barcroft manometer was filled up with the mixed gas; by repeating this procedure several times, complete anaerobic condition was successfully obtained.

For the measurement of RQ, indirect manometric method was used. In the case of CO_2 estimation, 3N H_2SO_4 was tipped from side arm after 60 minutes of incubation.

Oxygen uptake was always estimated by manometery.

Consumption of glucose was determined by the following method; bacterial suspension was incubated with the solution of glucose and phosphate buffer (pH 7.2) for 2 hrs. at 30° in an atmospher of N_2 and CO_2 in a ratio of 9 to 1. The bacterial cells were removed by centrifugation, and glucose in the supernatant was determined by Hanes method and the amounts of the sugar consumed were calculated.

Products of glucose metabolism were detected by paper chromatography. The bacterial suspension was incubated with glucose and phosphate buffer (pH 7.2) in both final concentration of M/30 for 17 hrs. at 30° under either aerobic or anaerobic condition. After incubation, the reaction mixtures were filtrated through Seitz's filter and the cell-free filtrate was used for the detection of volatile and non-volatile acids.

For the detection of volatile acids, the acidic solution was distilled by steam, then the distilled solution was converted to hydroxamic acid by the addition of NH_2OH, and this was used as the sample for paper chromatography. As solvent, ethanol/ammonia/water (80 : 5 : 15) was used and ethanolic solution of ferric chloride was sprayed as indicator. Non-volatile acids were detected by paper chromatography by using the extract which had been obtained from the reaction mixture with acidic ether for 48 hrs.. In this case, ethanol/ammonia/water or *n*-butanol/formic acid/water (4 : 1.5 : 1) and bromocresol green were used as solvent and indicator, respectively.

Results

(1) Growth and Comparison of Oxidative Activities.

The results previously reported[4] are shown in Fig. 1 and Table 1. As shown in Fig. 1, after a lag phase of about one hour, the average cell number was increased

Table 1. Oxidative activity towards several substrates.

	Substrate	Time of growth (min.) 0	10	20	30	60	120	180	300
Q_{O_2}	L-Leucine	421	243	214	129	165	364	409	322
	L-Glutamate	87	95	40	70	54	71	136	175
	Succinate	148	196	125	108	102	152	198	191
	Acetate	24	15	10	15	15	11	—	—
	Glucose	65	37	34	29	31	56	88	48
	Endogenous	3	11	—	10	0	0	0	0
Q_{MB}	Pepton-broth	2.08	—	—	0.59	0.72	1.14	2.22	3.64

Cited from Iimura *et al.*[4].

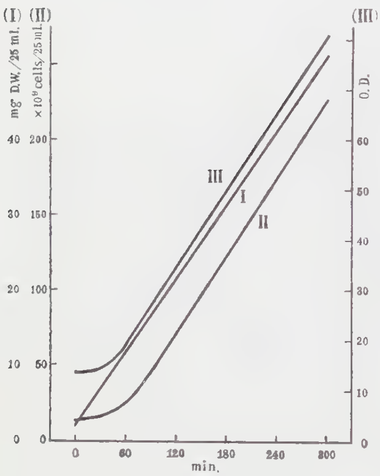

Fig. 1. Growth Curve.
I. Dry weight of cells.
II. Numbers of cells.
III. Optical density.
Cited from Iimura *et al.*[4].

exponentially. From the measurement of oxidative activities towards various substrates during these growth stages, it was discovered that the activity in most of the cases passes through a minimum at an incubation time of about 30 minutes, to show a gradual increase with the further lapse of time (see Table 1). The activity of dehydrogenase in bacterial cells was also measured in the presence of pepton-broth as hydrogen donor. As shown in Table 1, the level of methylene blue reduction was also lowest at a period of thirty minutes of growth.

(2) Measurement of Fermentative Activity.

It is evidently shown in Table 2 that the bacterial cells harvested at the lag phase of growth have a low fermentative activity as was the case with the oxidative metabolism; the bacterial cells cultivated for 120

Table 2. Fermentative activity of resting cells harvested at various growth stages of *P. vulgaris*.

Time of growth (min.)	0	10	20	30	40	50	60	90	120	180	240	300
$Q_{CO_2}^{N_2}$	74	26	41	62	65	65	63	81	143	114	115	98
Glucose consumed μg./mg. dry weight per 2 hrs.	303	—	—	251	—	—	266	—	409	399	389	351

Reaction mixture for measurement of $Q_{CO_2}^{N_2}$: Bacterial suspension (in KRB solution), 2 ml.; M/5 glucose, 0.5 ml.; H_2O, 0.5 ml. Experimental condition of glucose consumption, see text.

minutes show the highest activity. Similar trends of activity change were discovered also from the results of determination of glucose consumption under anaerobic condition, the rate being low in the lag phase cells as compared with that of the logarithmic phase cells (Table 2). In both cases the steep rise in activity that occurs in a period between 90 and 120 minutes of incubation is perhaps worth mentioning.

(3) Measurement of Respiratory Quotient.

In the next place, attempt was made to follow the level of oxidative output of CO_2 with glucose as the substrate, because in the present study the cultivation of bacterium was carried out under aerobic condition. As presented in Table 3, the RQ values were about unity at every phase of growth.

Table 3. Respiratory quotient measured with resting cells of *P. vulgaris* harvested at various growth stages.

Time of growth (min.)	0	10	20	30	40	50	60	120	180	240	300
RQ	1.08	0.85	1.08	0.97	1.09	1.07	1.04	1.02	1.04	1.10	1.07

Conditions of measurement: Final concentration of glucose and phosphate buffer (pH 7.2) M/30 and M/25 respectively; temperature, 30° (see text).

(4) Oxidation of Glucose.

To examine whether the mode of utilization of glucose by the bacterium should be altered in some stages of growth, estimates were made of the total oxygen uptake in the presence of M/1000 glucose. Table 4 reveals the fact that there are only certain insignificant variations in the rate of oxygen consumed through various stages of growth.

Table 4. Oxidation of glucose.

Time of growth (min.)	0	10	30	60	120	240
Total O_2 uptake (μl.)	198	190	212	195	197	179
Theoretical value (μl.)			373			

Conditions of measurement: Glucose M/1000; phosphate buffer (pH 7.2) M/500. Temperature, 30° (see text).

If an amount of M/1000 glucose was to be completely oxidized, theoretically a volume of 373 μl. O_2 should have been consumed, so the oxygen consumed practically in this experiment amounts to about 50 per cent of theoretical value.

(5) The Effect of Various Inhibitors on the Oxidation of Glucose.

The inhibitory effect of representative inhibitors including CN, NaF, AsO_2 and MIA, on the rate of oxidation of glucose by the bacterial cells was also investigated No significant difference was discovered when the cells at various growth phases were compared with this respect (Table 5). Therefore, it will be inferred that there

Table 5. Effect of inhibitors on glucose oxidation.

Inhibitor	Time of growth (min.)					
	0	10	30	60	120	240
	Inhibition (%)					
KCN (M/1000)	76	81	83	74	72	74
MIA (M/1000)	96	90	89	90	91	91
$NaAsO_2$ (M/1000)	54	47	50	51	37	68
NaF (M/500)	14	14	20	21	26	19

Conditions of experiment: Final concentrations; glucose M/30, phosphate buffer (pH 7.2) M/25, and temperature, 30°. Inhibitors were added at 0 time.

was no essential difference in the actual mechanism of glucose oxidation throughout the growth phases studied.

(6) Examination of Metabolic Products of Glucose.

If the pattern of glucose metabolism should be altered during the course of growth, the constitution of the resulting products must also be altered. In order to detect organic acids produced as a result of glucose fermentation by *P. vulgaris*, the reaction mixtures at appropriate stages of incubation were analyzed by paper chromatography. In this way spots for succinic and lactic acids were discovered in the chromatogram; lactic smaller in concentration as compared with succinic acid. Succinic acid was identified enzymatically, and lactic chemically. Another spot for an unknown volatile acid was obtained in the paper chromatogram. In all the growth stages studied, lactic and succinic acids and the above-mentioned unknown volatile acid were discovered in the fermentation mixture. No evidence for the formation of other organic acids was ever revealed. An experiment, on the other hand, was carried out to detect the formation, if ever, of organic acids under aerobic condition. Prolonged incubation of the organism with glucose for 4 and 7 hrs. unvariably gave negative results in this respect. A faint spot for succinic acid, however, was discovered in the chromatogram of reaction products of 2 hrs. incubation. This seems to suggest that the above-stated negative results in the 4 and 7 hour experiments were most plausibly caused by the further consumption of the substance once formed in the reaction mixture.

Discussion

As cited above, the activities of protein- and nucleic acid-biosyntheses, enzymic activities, resistance against antibial reagents, and adaptability of enzyme formation etc., have been proved to vary with the factor of the age of the population, whether it is young or aged.

When study was made of oxidative activities of glucose, amino acids, organic acids and pepton-broth as substrates, generally there could not be found any significant difference among these activities during the growth, except the cells in initial log phase, the activity of which was higher compared with other cells. As described in this paper, the fermentative activity and the rate of glucose consumption were found to rise at a period of 120 minutes incubation of bacterial culture. How-

ever, respiratory quotient and effect of respiratory inhibitors on glucose oxidation were found to change insignificantly when the cells harvested at any period of the bacterial culture were used. Therefore, energy yielding system seems not to change essentially through the course of growth. Further studies, however, are necessary to clarify the relationships between metabolic activities and growth phase.

It was demonstrated that *P. vulgaris* formed a considerable amount of succinic acid from glucose under anaerobic condition. As the mechanism of succinic acid formation under anaerobic condition, some enzymic systems can be considered. Among these systems oxidative condensation of two molecules of acetate as stated originally by Thunberg[5] is most probable. This reaction was demonstrated in *Aerobacter aerogenes* and in an extract of Tetrahymena[6]. Recently, it was also reported in pig heart muscle by D. D. Davies[7].

In the present study on *P. vulgaris*, it is presumed that succinate is formed by this condensing reaction, because the succinic acid was proved to be formed from pyruvate and ATP, but not from other possible intermediates of pathway of succinate formation[8]. To elucidate the mechanism of succinate formation conclusively, further detailed experiments are necessary.

Summary

Metabolic activities of *Proteus vulgaris* with glucose as substrate, were investigated with special reference to the influences of the age of the culture. Changes in activities of fermentation (together with the respiratory quotient), consumption of glucose, and organic acid formation from glucose, as well as the extent of inhibition of glucose oxidation by various respiratory inhibitors were followed, using bacterial cells in a state of "resting cell" suspension harvested at various stages of growth.

The fermentative activity and the rate of glucose consumption were found to be subject to a steep rise at a period between 90 and 120 minutes of incubation of bacterial culture which corresponds to the logarithmic growth phase. Otherwise, no significant change was ever discovered.

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摘　　要

Proteus vulgaris の生長過程におけるブドウ糖の代謝活性についてしらべた。醗酵能，呼吸率，ブドウ糖消費率，[illegible]などの種々の呼吸阻害剤による[illegible]糖酸化に対する[illegible]を，生長の各時期で得られた休止菌を用いて比較検討した。

醗酵能およびブドウ糖消費率は，生長の対数期に相当する 90 分から 120 分培養した菌で，急激に増加することがみられた。[illegible]を通じ有意な[illegible]はみられなかった。

Correction

by Hiroyuki HIROSE*

In the thesis titled "Rearrangement of the systematic position of a thermal alga, *Cyanidium caldarium* (Tilden) Geitler" which was published on the present magazine vol. 71, no. 844, pp. 347-352 in Oct. 1958, the author concluded that *Cyanidium caldarium* which belonged to Cyanophyceae should be transferred to Rhodophyceae, and also he gave this alga a new name, *Rhodococcus caldarius* (Tilden) Hirose. Later, however, he noticed that this name was contrary to the International Code of Botanical Nomenclature. Hereby the author wishes to declare that *Rhodococcus caldarius* (Tilden) Hirose is an illegitimate name under the code and the correct name for this alga is *Cyanidium caldarium* (Tilden) Geitler.

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Structure and Development of Plastids in Variegated Leaves*

by Rikizo UEDA** and Masaru WADA**

植田利喜造**・和田 優**: ふ入り葉におけるプラスチッドの構造と発達

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Since the pioneering work of Dalitzsch (1886) had appeared, several investigations have been made concerning the structure of plastids in variegated leaves (Randolph, 1922; Yasui, 1929; Zirkle, 1929; and some other workers). However, during recent two decades, no communication has been presented on this subject, until Strugger and Losada-Villasante (1956) published the detailed studies on plastids using variegated leaves of *Chlorophytum comosum* forma *medio-albinata*). According to their observation, albicated plastids found in variegated leaves are characterized as follows: (1) devoid of pigment, (2) amoeboid in form, (3) small in size, (4) vacuolate in structure, and (5) small in granum number.

In characterizing the albicated plastids in variegated leaves, it is necessary to study the structure and development of albicated plastids in detail appearing in a variety of plant species. The present study deals with this problem using various variegated leaves belonging to the chlorophyll type***.

Materials and Methods

Materials used in this study comprise 41 species of wild and cultivated plants belonging to 22 families (Table 1). For studies on the development of plastids, *Agave americana* var. *variegata* Nichols and a variegated form of *Allium fistulosum* were used.

Sections of albicated and normal green tissues of variegated leaves were prepared by free hand technique. Observations were made with preparations, vitally or after staining with rhodamine B (0.1%) or Sudan III solution, using either light microscope or fluorescence microscope.

* A part of this study was presented at the 29th Annual Meeting of the Genetics Society of Japan held at Sapporo on Sept. 3–5, 1957.

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*** Hara (1957) classified the variegated leaves into four types: (1) chlorophyll type (deficiency of chloroyhyll), (2) air-space type (presence of airial space just below the epidermal layer), (3) epidermis type (peculiar epidermis), (4) pigment type (occurrence of pigment other than chlorophyll).

Results

I. Structure of albicated plastids appearing in mature variegated leaves.

In order to see whether or not the albicated plastids in variegated leaves of various plants show five characteristics mentioned above, their color, form, size, vacuolization and number of grana were carefully observed under the microscope, in parallel with those of normal green chloroplasts side by side. In addition, fluorescence and Molisch's reaction (reduction of silver nitrate) of these two types of plastid were examined. In many plants investigated, it was found that the normal chloroplasts in green portion of mature variegated leaves were green, disk-shaped (ca. 5μ in diameter), and contained 40 to 50 grana, but were not vacuolate in every case. They were red-fluorescent and reduced silver nitrate. On the contrary, a conspicuous difference was observed in the structure of albicated plastid within the plant species examined; the results are summarized in Table 1.

From the table below, albicated plastids can be classified into following five types as regards color, form, size and vacuole:

Type 1. Plastids being chlorophyll-deficient, amoeboid, small and vacuolate (Pl. IV: Figs. 7a–d).

In this type 21 species are included: *Thujopsis dolabrata* Sieb. et Zucc., *Juniperus chinensis* L. var. *procumbens* Endl., *Zelkova serrata* Makino, *Hydrangea macrophylla* Seringe var. *otaksa* Makino., *Camellia japonica* L., *Ajuga decumbens* Thunb., *Glechoma hederacea* L., *Plantago major* L. var. *asiatica* Decne., *Ligularia tussilaginea* Makino, *Arrhenatherum elatimus* Mert. et Koch var. *tuberosum*, *Phalaris arundinacea* L. var. *Picta* L., *Acorus gramineus* Soland., *Chlorophytum comosum* Baker, *Chlorophytum elatum* R. Br., *Hosta undulata* Bailey (forma *medio-albinata* and forma *albo-marginata*), *Allium fistulosum* L., *Aspidistra elatior* Blume, *Liriope graminifolia* Baker, *Agave americana* var. *variegata* Nichols, *Alpinica chinensis* Bosc.

Type 2. Plastids similar to normal green chloroplasts except their smaller size (Pl. III: Figs. 1a and b, 3a and b).

This type includes 4 species belonging to Pteridophyta: *Adiantum cuneatum* Lang. et Fisch., *Pteris cretica* L. var. *albolineata* Hook., *Pteris engiformis* Burm. var. *victoriae* Baher., *Pteris argyrea* Moore.

Type 3. Plastids which are not amoeboid but spherical or elliptic (Pl. III: Figs. 7, 8, 9a and 11b).

This type includes the following 11 species: *Polygonum persicaria* L., *Polygonum orientale* L., *Fagopyrum esculentum* Moench., *Sedum lineare* Thunb. var. *albomarginatum* Makino, *Kerria japonica* DC., *Elaeagnus pungens* Thunb., *Fatsia japonica* Decne et Planch., *Osmanthus ilicifolius* Stand., *Ipomoea* Nil Roth., *Erigeron philadelphicus* L., *Iris japonica* Thunb.

Type 4. Plastids which are not vacuolate (Pl. III: Figs. 1b, 2, 3b, 8, 9b and 10).

This type includes 13 species as follows: *Adiantum cuneatum* Lang et Fisch.,

Table 1. Characteristics of albicated plastids in mature variegated leaves

Characteristics of plastids / Plant	Color	Form	Average size (μ)	Number of grana* (average)	Vacuole	Fluorescence (red)	Reduction of $AgNO_3$	Remarks
Pteridophyta:								
Adiantum cuneatum Lang et Fisch.	green	amoeboid or spherical	2.4	10-20 (15)	−	+	+	Pl. III: Fig. 1, a and b.
Pteris cretica L. var. *albolineata* Hook. ([illegible])	〃	〃	3.5	10 13 (12)	−	+	+	Pl. I: Fig. 2.
Pteris engiformis Baurm. var. *victorias* Baher. (フイリイノモトソウ)	〃	〃	2.8	10-13 (12)	−	+	+	Pl. I: Fig. 3, a and b.
Pteris argyrea Moore	〃	〃	2.4	10-13 (12)	−	+	+	
Gymnospermae:								
Thujopsis dolabrata Sieb. et Zucc.	〃	〃	3.5	3 - 4 (4)	±	+	…	Pl. III: Fig. 4.
Juniperus chinensis L. (イブキ)	〃	〃	1.4	3 - 5 (4)	±	+	…	Pl. III: Fig. 5.
Juniperus chinensis L. var. *procumbens* Endl. (ソナレ)	〃	〃	1.4	7 -10 (9)	±	+	…	Pl. III: Fig. 1.
Dicotyledoneae:								
Zelkova serrata Makino (ケヤキ)	colorless	〃	3.0	1 - 3 (2)	+		·	
Polygonum persicaris L. (ハルタデ)	yellow	spherical	2.5	1 - 3 (2)	+	+	…	Pl. III: Fig. 7, a and b.
Polygonum orientale L. (オオケタデ)	colorless	〃	2.2	3 - 5 (4)	+	·	·	
Fagopyrum esculentum Moench. (ソバ)	colorless or pale green	〃	2.5	1 - 3 (2)	−	+	+	Pl. III: Fig. 8.
Sedum lineare Thunb. var. *albomarginatum* Makino (フクリンマンネングサ)	colorless	〃	7.5	1 - 5 (3)	−	·	·	
Hydrangea macrophylla Seringe var. *otaksa* Makino (アジサイ)	colorless or pale yellow	amoeboid or spherical	2.8	1 - 6 (4)	+	…	+	
Kerria japonica DC. (ヤマブキ)	pale green or yellow	spherical	5.5	1 -15 (8)	−	+	…	Pl. III: Fig. 9, a and b.
Pelargonium zonale Ait. (モンテンジクアオイ)	colorless or pale yellow	〃	5.0	1 - 8 (5)	−	…	…	Pl. III: Fig. 10.

Camellia japonica L. (ツバキ)	yellow or pale green	spherical	4.6	1-5 (3)	±	…	·	
Elaeagnus pungens Thunb. (ナワシログミ)	colorless or pale green		5.5	0-3 (2)	±	…	…	
Fatsia japonica Decne. et Planch. (ヤツデ)	colorless	〃	1.8	1-5 (3)	+	…	…	
Aucuba japonica Thunb. (アオキ)	pale green	amoeboid or spherical	1.6	3-5 (4)	±	…	…	Pl. III: Fig. 11, a and b.
Osmanthus ilicifolius Stand. (ヒイラギ)	colorless	spherical	3.2	0-5 (3)	+	…	…	
Ipomoea Nil. Roth. (アサガオ)	〃	〃	1.8	0-5 (3)	+	…	…	
Ajuga decumbens Thunb. (キランソウ)	colorless or pale green	amoeboid or spherical	2.[illegible]	5-10 (8)	±	−	?	Pl. III: Fig. 12.
Glechoma hederacea L. ([illegible])	〃		[illegible]	0-5 (5)	+	…	·	
Plantago major L. var. *asiatica* Decne. (オオバコ)	colorless	amoeboid	7.0	3-5 (4)	+	·	…	Pl. III: Fig. 13.
Erigeron philadelphicus L. (ハルジオン)	pale green	spherical	[illegible].3	5-10 (8)	−		·	
Petasites japonicus Miq. (フキ)	colorless or pale green	amoeboid or spherical	2.[illegible]	3-5 (4)	−		·	
Ligularia tussilaginea Makino (ツワブキ) "spot type"	colorless	amoeboid	2.2	0-5 (3)	+	…	…	Pl. III: Fig. 14, a and b.
"chimeric type"	〃	〃	2.8	0-5 (3)	+	·	?	
Monocotyledoneae:								
Arrhenatherum elatimus Mert. et Koch. var. *tuberosum* (リボングラス)	pale yellow	amoeboid or spherical	1.7	0-5 (3)	±		+	
Phalaris arundinaces L. var. *picta* L. (シマヨシ)	colorless	amoeboid	3.0	0-5 (3)	+	?	?	
Acorus gramineus Soland. (セキショウ)	〃	〃	3.2	3-5 (4)	+	…	…	
Tradescantia fluminensis Vell. (シロフハカタカラクサ)	pale yellow or pale green	amoeboid or spherical	4.0	3-5 (4)	−	−	±	
Chlorophytum comosum Baker (オリズルラン)	colorless or yellowish green	〃	2.7	1-5 (3)	+	…	+	**
Chlorophytum elatum R. Br. (ヒロハノオリズルラン)	〃	〃	2.7	1-5 (3)	+	+	+	** Pl. III: Fig. 15.

Hosta undulata Baily (スジギホウシ) "*medio-albinata*"	colorless or pale green	amoeboid	4.0	0 - 7 (4)	±	—	—	**
"*albo-marginata*"	〃	〃	4.0	0 - 7 (4)	±	±	±	** Pl. III: Fig. 16.
Hemerocallis fulva L. var. *Kwanso* Regel (ヤフカンゾウ)	〃	amoeboid or spherical	1.4	0 - 5 (3)	—	+	…	**
Allium fistulosum L. (ネギ)	〃	〃	3.4	0 12 (6)	±	±	…	**
Aspidistra elatior Blume (ハラン)	pale yellow or pale green	〃	1.8	3 5 (4)	±	±	·	
Liriope graminifolia Baker (ヤブラン)	colorless or yellowish green	〃	4.0	0 - 5 (3)	+	+	…	Pl. III: Fig. 7, a and b.
Agave americana var. *variegata* Nichols (リュウゼツラン)	colorless or pale green	〃	3.2	0 -15 (8)	±	+	+	Pl. IV: Fig. 7, a and b.
Iris japonica Thunb. (シャガ)	colorless or yellow	spherical	2.4	0 -10 (5)	—	+	+	
Alpinica chinensis Bosc. (フイリゲットウ)	colorless	amoeboid or spherical	4.6	1 - 3 (3)	±	±	·	

* The term 'garna' designates the granules which are somewhat bigger than or nearly same to the grana of normal chloroplasts. They are colorless or light green and are capable of vital staining with rhodamine B or Sudan III solution.

** Albicated plastids and green chloroplasts were mixed in a cell.

Pteris cretica L. var. *albolineata* Hook., *Pteris engiformis* Burm. var. *victoriae* Baher., *Pteris argyrea* Moore, *Fagopyrum esculentum* Moench., *Kerria japonica* DC., *Pelargonium zonale* Ait., *Aucuba japonica* Thunb., *Erigeron philadelphicus* L., *Petasites japonicus* Miq., *Tradescantia fluminensis* Vell., *Hemerocallis fulva* L. var. *Kwanso* Regel., *Iris japonica* Thunb.

Type 5. Plastids, the size of which is bigger than or nearly equal to that of the normal chloroplast.

This type includes the following 4 species: *Sedum lineare* Thunb. var. *albomarginatum* Makino, *Kerria japonica* DC., *Camellia japonica* L., *Elaeagnus pungens* Thunb.

In consequence, it is recognized that conspicuous difference exists from plant to plant as regards the structure of albicated plastid, whereas the number of grana is commonly small irrespective of plastid-types, as seen from Table 1. Namely, 40–50 grana were present in normal chloroplast, while 0-20 in albicated plastids (Pl. III: Figs. 1-17). Besides, it is noteworthy that the slightest amount of chlorophyll is detectable under the fluorescence microscope in any of the albicated plastids (Table 1). Molisch's reaction was also shown to be positive in albicated plastids almost to the same extent as in the normal chloroplasts.

As denoted in Table 1 (marked with **), albicated plastids and chloroplasts were often found to be present admixed in a single cell in some liliaceous species.

II. Development of albicated plastids in variegated leaves.

Although the structural development of normal green chloroplasts was frequently studied by several authors (Düvel, 1954; Fasse-Franzisket, 1955; Grave, 1954; Mühlethaler, 1955; Strugger and Perner, 1956, and others), the development of albicated plastids has been studied rarely (Randolph, 1922; Zirkle, 1929; Stubbe and Wettstein, 1955; Strugger and Losada-Villasante, 1956).

In the present study *Agave americana* var. *variegata* Nichols and a variegated form of *Allium fistulosum* L. were used. In these plants leaf blade is longitudinally divided into white and green portions in mature leaves, but this is not conspicuous in younger ones. For the sake of convenience, an entire blade of young leaf (ca. 10 × 3 cm. in *Agave*, and ca. 5 × 2 cm. in *Allium*) was divided into five zones from base to top of a leaf as follows:

Zone I: The basal *viz.* the youngest part of a leaf. In this zone all parts are still colorless.

Zone II: Normal part of this zone is pale yellow and albicated part colorless.

Zone III: This zone includes a middle portion of a leaf. Normal part of this zone is pale yellowish green and albicated part pale yellow.

Zone IV: Normal part of this zone is pale green and albicated part pale yellowish green.

Zone V: This zone lies at the top *viz.* mature part of a leaf. Normal part is dark green and albicated part yellowish green.

Both in *Agave* and *Allium*, corresponding leaf zones gave similar results as follows:

Zone I: No structural difference was observed between normal and albicated plastids. They were colorless, and spherical or amoeboid in form, average size of which was 0.8 to 1.6μ in diameter. They contained only one granum, and sometimes none (Pl. IV: Fig. 1). Red fluorescence characteristic of chlorophyll was not observed in both plastids. After staining with rhodamine B, they exhibit pale yellow fluorescence.

Zone II: No structural difference between normal and albicated plastids; they were colorless, spherical or amoeboid in form, and 1.2-2.4μ long in diameter. They contained none to 6 grana which fluoresced in red under the fluorescence microscope (Pl. IV: Figs. 2 and 3).

Zone III: Plastids in normal part were amoeboid in form and 2.0-4.0μ long in diameter. Each of them contained 10-25 grana. On the other hand, albicated plastids were 1.8-3.2μ long in diameter and, in general ca. 10 grana were present in each, while some were free from granum. Moreover, in some of the albicated cells it was found that a single cell contains several plastids having one minute vacuole (0.8μ in diameter) in each. But this was not the case in normal plastids (Pl. IV:

Figs. 4a and b).

Zone IV: Plastids in normal part were more or less amoeboid and 3-5μ long in diameter. They contained a large number of grana (Pl. II: Figs. 5a and b). Albicated plastids were amoeboid or sometimes rod-like (5×1.6μ) in shape. The number of grana in a single albicated plastid was almost same (ca. 10 grana) as that found in zone III. Several minute vacuoles were found in a single albicated plastid (Pl. IV: Fig. 6).

Zone V: Plastids in normal part were green and 4.5-6μ long in diameter. Albicated plastids were almost same in microscopic appearance as observed in zone IV (Pl. VI: Figs. 7a and b), except that the vacuoles in some plastids were found to be larger (1.2-1.6μ in diameter), as seen in Pl. IV: Figs. 7c and d.

From the observations mentioned above, it is seen that between the normal and albicated plastids no structural difference exist at their initial stage of development, whereas this is gradually brought about in accord with the growth of plastids. For instance, an increase in number of grana gradually occurs in normal plastids, as shown in Figs. 1 and 2, whereas in the case of albicated plastids neither increase nor new formation of grana is occasionally observed during the course of plastid development (1 in zone III and V in Fig. 2). Vacuole is sometimes formed only in albicated plastids, and is capable of growing so large as to bring about an enlargement of the albicated plastid as a whole.

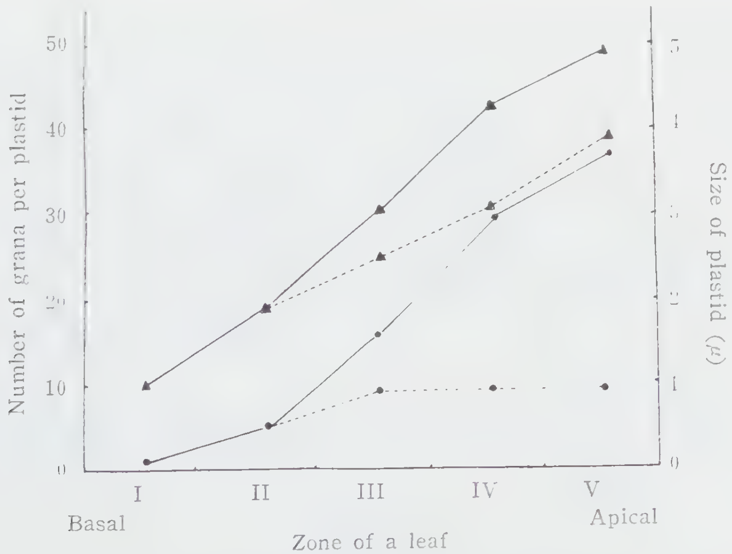

Fig. 1. Comparison of the number and size of albicated plastids with those of normal chloroplasts in *Agave americana* var. *variegata* Nichols.

—▲—: Average size of normal chloroplast.
—●—: Average size of albicated plastid.
--▲--: Average number of grana in a single normal chloroplast.
--●--: Average number of grana in a single albicated plastid.

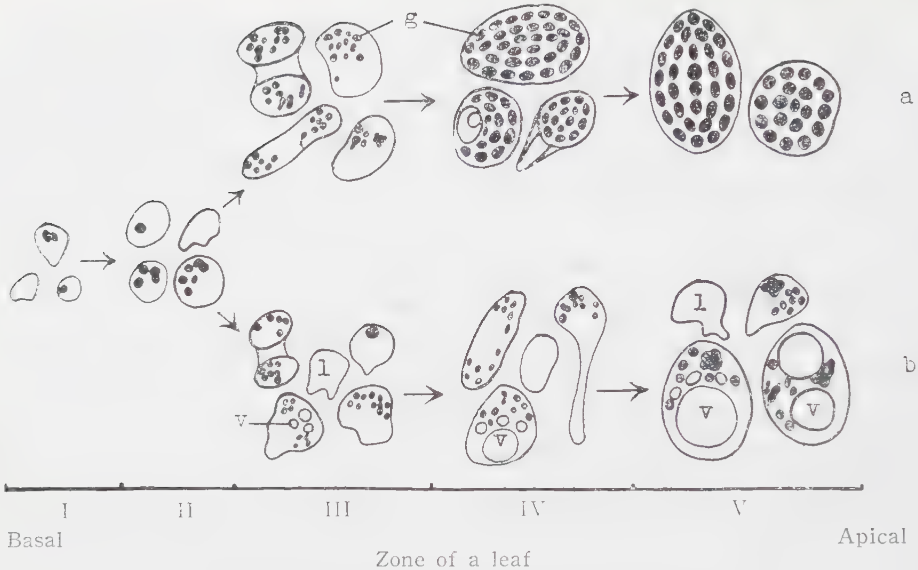

Fig. 2. Developmental relationship between normal and albicated plastids in *Agave americana* var. *variegata* Nichols.
(a : normal part, b : albicated part, g : granum, l : non-granum plastid, v : vacuole)

Discussion

Recently, Strugger and Losada-Villasante (1956) pointed out some characteristics of albicated plastid : deficient in chlorophyll, amoeboid in form, small in size and vacuolate in structure. In our examination of albicated plastids in 21 out of 41 species of variegated plants studied, all these characteristic points were clearly demonstrated (cf. type 1). However, there were several exceptions as follows :

(1) In some variegated ferns, chlorophyll is not always deficient in the plastids from variegated tissues. Due to the smallest number and size of plastids, variegation comes into appearance in these ferns (cf. type 2).

(2) In albicated plastids, there are many which are spherical, but not amoeboid (cf. type 3), as seen in *Polygonum persicaria* L., *Polygonum orientale* L., *Fagopyrum esculentum* Moench., etc.

(3) There are several albicated plastids which are not vacuolate (cf. type 4). For example : *Adiantum cuneatum* Lang et Fisch., *Pteris cretica* L. var. *albo-lineata* Hook., *Pteris engiformis* Burm. var. *victoreae* Baher., etc.

(4) We cannot always attribute the small size to the albicated plastids, because they are quite large (cf. type 5) in the following plants : *Sedum lineare* Thunb. var. *albomarginatum* Makino, *Kerria japonica* DC., *Camellia japonica*, L., *Elaeagnus pungens* Thunb.

(5) Some albicated plastids in *Kerria japonica* DC., *Elaeagnus pungens* Thunb., etc. are not amoeboid, and neither vacuolate nor small, although they are deficient

in chlorophyll.

In view of these facts, the form, size and vacuolisation cannot be taken as essential characteristics for albicated plastids. On the other hand, the number of grana in a single plastid was always smaller in the albicated than in the normal, as described in *Chlorophytum* by Strugger and Losada-Villasante (1956). Besides, some of the albicated plastids were found to be free from granum in certain plant species studied (cf. Table 1). The smallest number of granum, *viz*. the abortion of plastid structure, results in chlorophyll-deficiency in albicated plastids, since it has been shown in higher plants that the chlorophyll is mainly formed in grana (Düvel and Mevius, 1952; Strugger, 1954 and Düvel, 1954). This is confirmed again from the present observation made on albicated plastids at various developmental stages using *Agave americana* var. *variegata* Nichols and a variegated form of *Allium fistulosum* L.

Yasui (1929) observed, for the first time, several cells having different types of plastids in variegated leaves of *Hosta japonica*. We could also observe cells with mixed plastids in *Chlorophytum comosum* Baker, *Chlorophytum elatum* R. Br., *Hemerocallis fulva* L. var. *Kwanso* Regel and a variegated form of *Allium fistulosum* L. (cf.** in Table 1). These findings seem important for our further investigation of the plastid individuality.

Summary

1. Structure of plastids was investigated using variegated plants of 41 species belonging to 22 families. For the study of plastid development, *Agave americana* var. *variegata* Nichols and a variegated form of *Allium fistulosum* L. were employed. The results were summarized in Table 1 and Figs. 1 and 2.

2. Albicated plastids account for the structural abortion. The number of grana in albicated plastids is remarkably small in comparison with that of the normal green chloroplasts. Accordingly, albicated plastids are usually devoid of chlorophyll.

3. In *Agave* and *Allium*, the granum in albicated plastid ceased to multiply at an earlier stage of development.

4. Molisch's reaction is found to be positive in all albicated plastids as well as in normal green chloroplasts.

5. In some species of Liliaceae, albicated plastids and green chloroplasts were present admixed in a single cell.

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Explanation of Plates

Plate III. Microphotographs of normal green chloroplasts and albicated plastids in mature leaves. (Figs. 15 and 16: ×1,200. Others: ×3,000. Figs. 1a and 9a: Stained with Sudan III. Others: Stained with rhodamine B. P in fig. denotes plastid).

Fig. 1. *Adiantum cuneatum* Lang et Fisch. 1a: normal green chloroplasts. 2b: albicated plastids.

Fig. 2. *Pteris cretica* L. var. *albo-lineata* Hook: albicated plastids.

Fig. 3. *Pteris engiformis* Burm. var. *victoriae* Baher: 3a: normal green chloroplasts. 3b: albicated plastids.

Fig. 4. *Thujopsis dolabrata* Sieb. et Zucc.: albicated plastids.

Fig. 5. *Juniperus chinensis* L.: albicated plastids.

Fig. 6. *Juniperus chinensis* L. var. *procumbens* Endl.: albicated plastid.

Fig. 7. *Polygonum pericaria* L.: albicated plastids. 7a: vacuolated plastids. 7b: non-vacuolated plastids.

Fig. 8. *Fagopyrum esculentum* Moench: albicated plastids.

Fig. 9. *Kerria japonica* DC.: 9a: normal green chloroplasts. 9b: albicated plastids.

Fig. 10. *Pelargonium zonale* Ait.: albicated plastids.

Fig. 11. *Osmanthus ilicifolius* Stand. 11a: normal green. 11b: albicated plastid.

Fig. 12. *Ajuga decumbens* Stand.: albicated plastids.

Fig. 13. *Plantago major* L. var. *asiatica* Decne.: albicated plastids. 13a and b: vacuolated plastid.

Fig. 14. *Ligularia tussilaginea* Makino: albicated plastids. 14a: non-vacuolated plastids. 14b: vacuolated plastids.

Fig. 15. *Chlorophytum elatum*: albicated plastids.

Fig. 16. *Hosta undulata* Bailey (*albo-marginata* form): cell containing colorless and green plastids. (g: green plastid. 1: colorless plastid. v: vacuolated plastid).

Fig. 17. *Liriope graminifolia* Baker. 17a: normal green chloraplasts. b: albicated plastids.

摘　　要

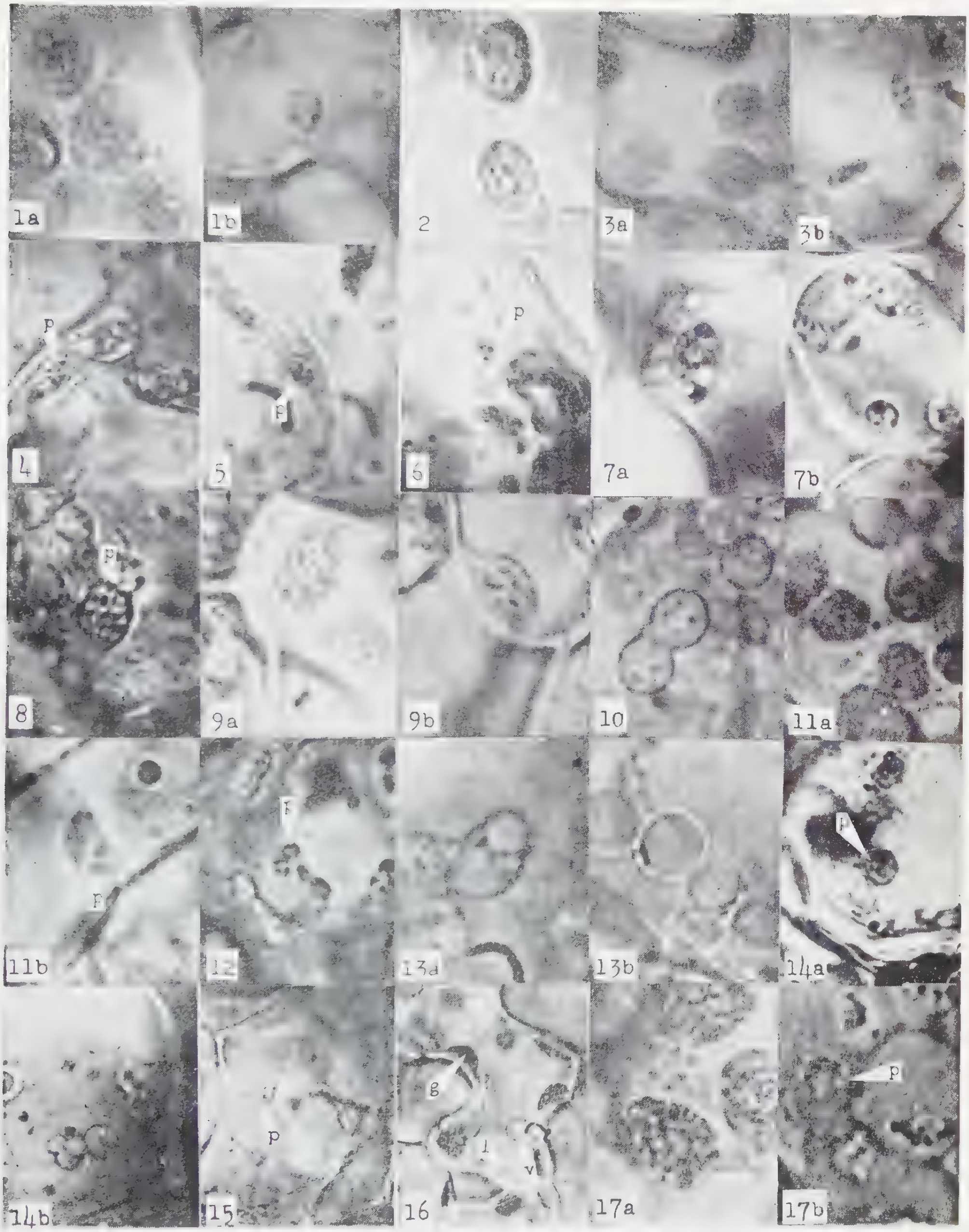
1. ふ入り葉のプラスチッドの特性として Strugger and Losada-Villasante (1956) は，オリズルランを用いて，(1) 色素が少ないこと，(2) 形がアメーバ状であること，(3) 大きさが小さいこと，(4) 空胞を有すること，(5) グラナの数が少ないことをあげている。我々は 41 種のふ入り植物について上記の特性を検討した結果，植物の種類によってかなりの相違があり，上の 5 つの条件を全部満足するものは 41 種の植物中 21 種だけで，他は不満足であった。

2. しかしいずれのふ入り葉のプラスチッドでも，正常の葉緑体に比して，グラナの数は常に少なかった。このことから，ふ入り葉のプラスチッドにおける葉緑素欠乏はグラナの数の少ないことに起因すると考えられる。

3. このことを確めるために，リュウゼツランとネギを用い各発達段階を追って正常の葉緑体とふ入り葉のプラスチッドの構造について比較観察を行なった。その結果，ふ入り葉のプラスチッドではグラナの増加は発達の初期で止ってしまうことがわかり，上に述べたことの正しいことが確認された。

4. Molisch 反応はふ入り葉のプラスチッドでも，程度の差はあるが，すべて陽性であった。

5. ユリ科の数種のふ入り植物では 1 細胞中に無色のプラスチッドと緑色の葉緑体を混在していた。



R. Ueda and M. Wada: Structure and Development of Plastids in Variegated Leaves.

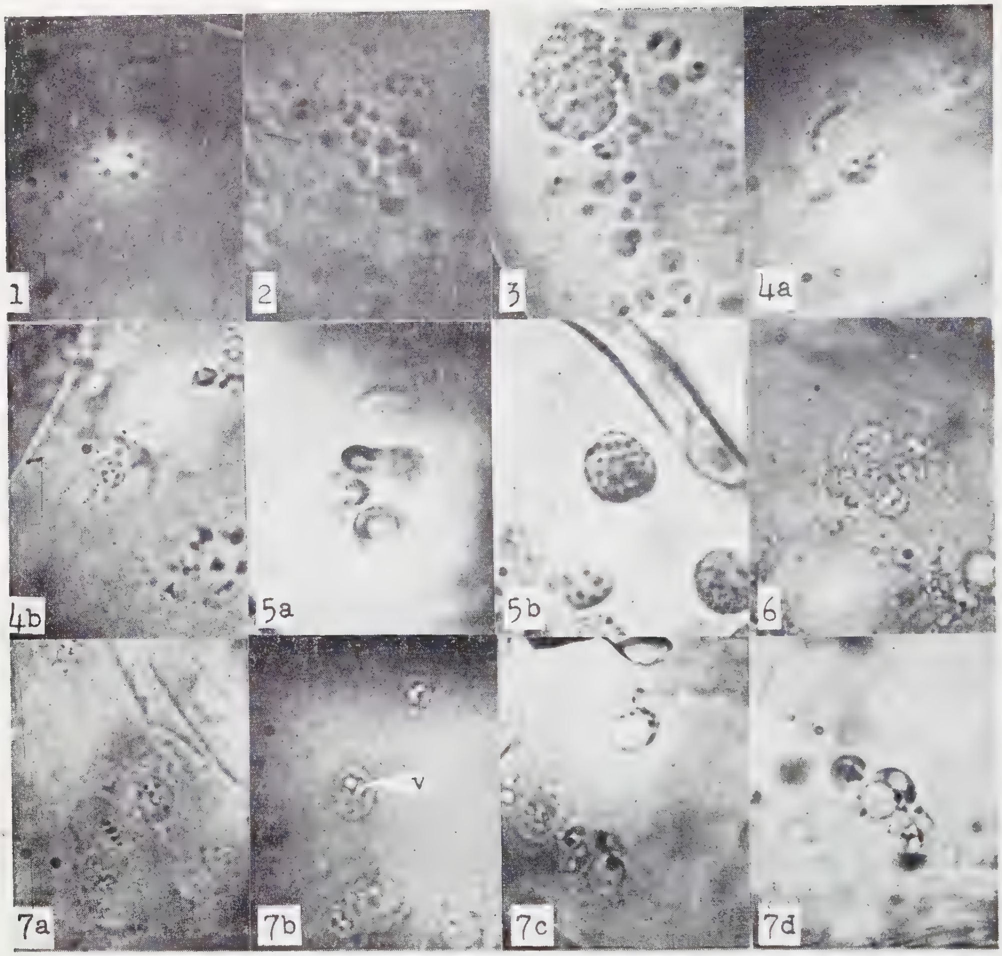


Plate IV. Development of normal green chloroplasts and albicated plastids in *Agave americana* var. *variegata* Nichols, stained with rhodamine B. ×2, 000.

Fig. 1. Zone I of a leaf: structural difference is not clear between normal green chloroplasts and albicated plastids. Figure shows proplastids in green tissues.

Fig. 2 and 3. Zone II: the same as in zone I.

Fig. 4. Zone III: a: normal green chloroplasts. b: albicated plastids.

Fig. 5. Zone IV: young normal chloroplasts. a: chloroplasts with colorless stroma. b: somewhat mature chloroplasts.

Fig. 6. Zone IV: albicated plastids with small vacuoles.

Fig. 7. Albicated plastids in zone V: a: amoeboid plastids. b: amoeboid and vacuolated plastids (v: vacuole). c and d: spherical and vacuolated plastids, respectively.

R. Ueda and M. Wada: Structure and Development of Plastids in Variegated Leaves.

Studies on the Growth of Fruit Body of Fungi I. Existence of a Hormone Active to the Growth of Fruit Body in *Agaricus bisporus* (Lange) Sing*

by Hiroshi HAGIMOTO** and Michio KONISHI**

萩本　宏**・小西通夫**：菌類子実体の生長に関する研究 I.
ツクリタケ（西洋マツタケ）子実体の生長ホルモンの存在

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Studies on the growth hormones in lower plants are behind in comparison with those of higher plants. Buller (1934)[1] introduced an idea of growth hormone to explain heliotropism of sporangiophore in a phycomycete, *Pilobolus*. Borriss (1934)[2] claimed the existence of a growth hormone in *Coprinus lagopus*. Development of fruit body was stopped by the removal of pileus at early stage. It was concluded that hormone occurring in the pileus acts on the growth zone of the stipe. This is the first report with experimental evidence on the growth hormone active to fruit body. But he gave up further study. Recently Urayama[3] (1956) also stated the existence of hormone in fruit body of *Agaricus campestris* (=*A. bisporus*). The present study began in spring of 1957. It has established evidences for the existence of a hormone active to the growth of fruit body.

Materials and Methods

The experiments were performed at mushroom flats constructed in the dark rooms of the Morimoto Mushroom Nursery at Momoyama in Kyoto, Japan.*** As the materials, the white strain of *Agaricus bisporus* (Lange) Sing.**** was used, and sometimes the snow white strain. About 30 mm. long mushrooms were used for the material. They were prepared for experiments after the "pre-treatment," at which the parallel sides of fruit body were cut off symmetrically at the equal distance from the central axis of the stipe (Fig. 1). The pre- and experimental treatments were performed under the illumination of artificial light. The fruit body of *A.*

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*** The authors are indebted to Mr. H. Morimoto, the owner, for generous accomodation in culture flats.

**** *Agaricus bisporus* (Lange) Sing., the common commercial mushroom has two spores on each basidium. *A. campestris* Fr. is a synonym of *A. bisporus* in many cases.

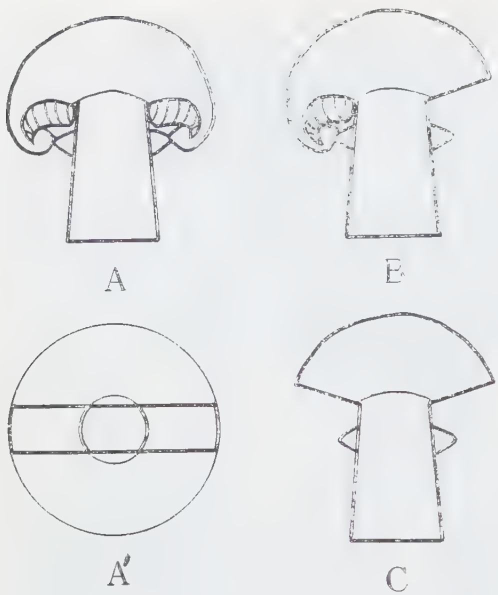

Fig. 1. Procedure of the fundamental treatment. A, A′: Cutting off the parallel side (A′: Top view). B: Removal of the gills on one side. C: Removal of the whole gills (used as control).

campestris (= *A. bisporus*) has been reported by Buller (1905)[4] to grow quite normally in both sunlight fields and completely darkened cellars. The fruit body is indifferent to light but shows negatively geotropical response. The pre-treatment did not disturb the normal growth of fruit body, at least during the experiments, except the cases of younger stages or inadequate conditions such as excessively high temperature or insufficient moisture.

The pre-treated mushroom has two symmetrical planes crossing at right angle through the central axis of the stipe. After the pre-treatment, the mushroom becomes flattened and the pileus has gills only at the right and the left side of the cut surface. The results of gill- or pileus-removing treatments thus appear as a curvature of the stipe. The curvatures were recorded usually five days after the treatments.

For the histological work, the material was fixed in formalin-acetic acid-alcohol mixture for more than 24 hours, then immediately transferred to 70% alcohol without washing, dehydrated with alcohol and paraffin-sectioned in 8 μ, and stained with Delafield's haematoxylin.

Observations and Results

A. Growth behavior of the fruit body.

Stipe growth after reaching a certain length takes place mainly at the growth zone, namely a narrow part just beneath the pileus and above the ring[2,5),6),7),8)]. This growth seems to depend only upon cell elongation: The degree of cell elongation at growing zone measured under microscope with sectioned material, is roughly parallel to that of growth of fruit body (Fig. 2). Bonner *et al.* (1956)[8] reported that there was little or no increase in cell number in the stipe after about 18 mm. stage in the fruit body of snow white strain of *A. campestris* (= *A. bisporus*). The material mushrooms used for the experiment, about 30 mm. long, may therefore be in the stage of rapid growth depending only on cell elongation, at least in the stipe. The expansion zone of pileus seems to exist near the margin as already reported by Bonner *et al.*[8].

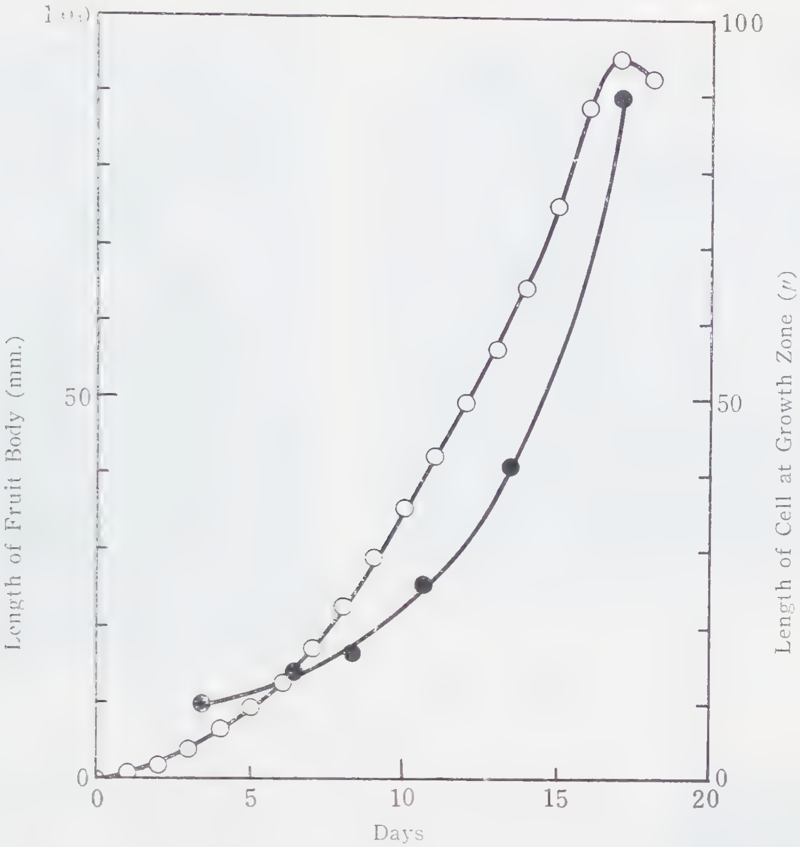

Fig. 2. *A. bisporus.* Growth curve of fruit body (○) and cell length in the growth zone (●). Averages of 30 measurements. Temperature: 11.0–23.6° (cellar), 14.2–19.5° (10 cm. depth of the culture bed). Relative humidity: 83–94%.

B. Evidences for the existence of growth substance in fruit body.

Urayama (1956)[3] reported that the stipe curved to the gill-free side when half of the gills were cut off from the pileus. After this fact was ascertained, further experiments were done to confirm that this curvature was neither due to the cutting nor to transpiration from the cut surface: When whole gills on one side and the corresponding region of the pileus surface on the other side were cut off, the stipe curved also to the gill-free side (Photo. 1). When gills were removed carefully on one side with remaining smaller amounts of gills near the stipe so as not to wound the growth zone, or if the different amounts of gills were cut off from both sides of pileus, the stipe curved to the gill-few side. Also only the pileus on the gill-abundant side grows and expands normally. The resulted curvature may only connect with the existence of gills. Not only the elongation of the stipe but also the pileus expansion itself seems to depend on the presence of the gills. When whole gills were removed, the stipe elongated only a little and pileus did not expand, owing to removal of the pileus margin at which most expansion is to be carried out; some times these fruit bodies withered up. However, the withering was

Photo. 1. Stipe curvatures. Parts of pileus-flesh on the gill-sides are cut off at right angle (left) or triangular shaped (right) at young stage. Arrows show the operating parts.

Photo. 2. A mica plate (➡) inserted between pileus-flesh and stipe on one side of fruit body.

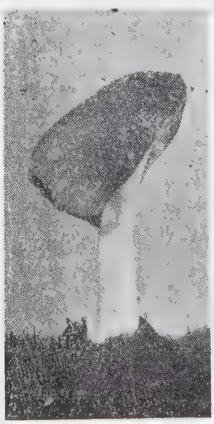

Photo. 3. Curvature caused by the half-removal of pileus of *Coprinus radians* at the young stage.

prevented when only a small amounts of gills remained, and further growth took place. Other experiment also supports this fact (Fig. 3); with a gap the pileus was divided into two parts, the smaller part with gills and the remainder, the larger parts without gills. The former could live long and grew remarkably whereas the latter and the adjoining part of stipe withered in many cases. The above-mentioned phenomena suggest that the gills supply some growth promoting substance to the growth zone of the stipe.

A mica plate was inserted between pileus-flesh and stipe on the one side of the pre-treated fruit body. Bending of the stipe to the mica side was resulted; the interruption of the streaming of stimulator from the gills may be the cause of this bending (Photo. 2). When, in addition, the gills of the mica-free side were cut off, the stipe grew considerably but did curve scarcely. Some mollification of asymmetric distribution of growth substance may be the cause of this phenomenon.

The curvature of the stipe was also found to occur in the case when the gills were detached from the pileus, connecting with the stipe only through the partial veil, as shown in Fig. 4.

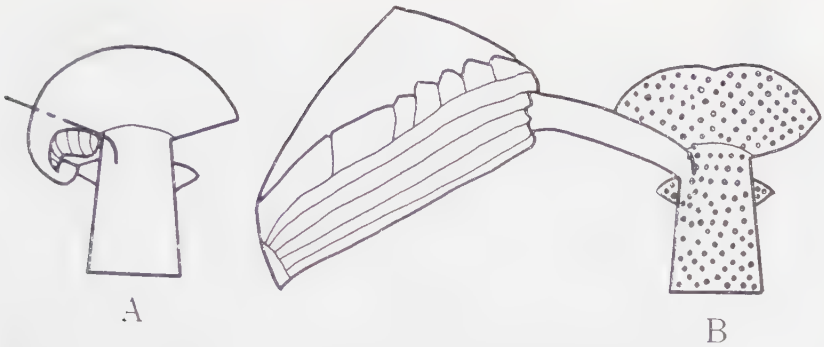

Fig. 3. Growth of a part of fruit body connected with a piece of gills, showing the relation between growth of fruit body and existence of gills. A: Method of treatment (chain line: A notch). B: Result caused by the treatment (dotted portion withered).

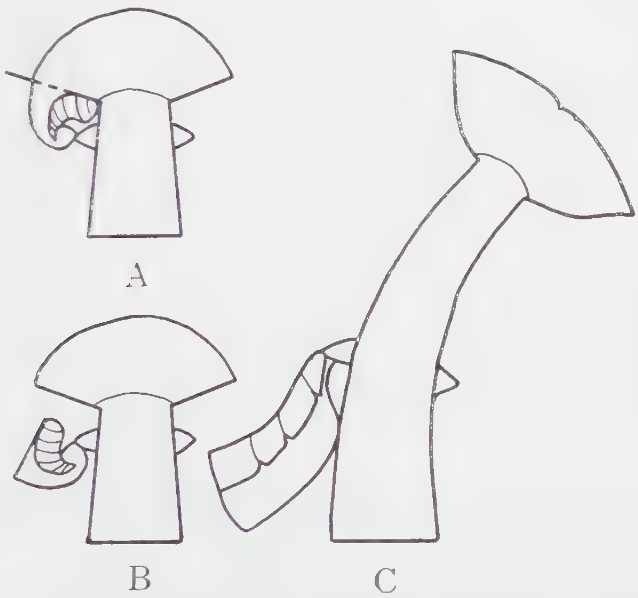

Fig. 4. Experiment showing transportation of growth substance through partial veil from gills to stipe. A and B: The cut procedure. The pileus is cut off from the other part except a connection only through partial veil along a notch (chain line). C: Curvature caused by the treatment.

The vertical mica plate insertion into the center of pileus was kept short to avoid injury of the growth zone (Fig. 5-A). This treatment afterwards split both the pileus and stipe open and divided then into two flanks (Fig. 5-C). Similar treatment applied to intact fruit body (Fig. 5-B) led to the same split as in the treated fruit body. Insertion of crossed mica plates (Fig. 5-D), splitted the fruit body open into four parts. A disproportionate distribution of growth substance between the surface and the center of stipe may be the cause of these phenomena.

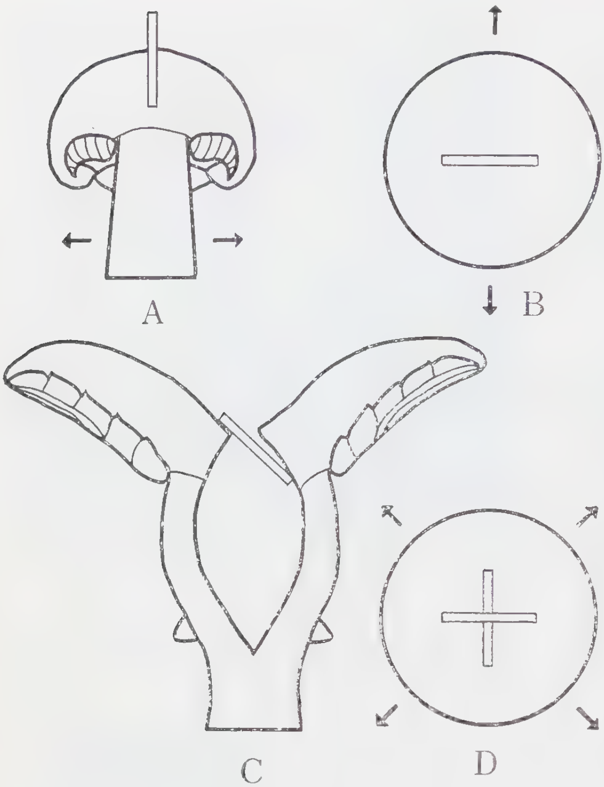

Fig. 5. Methods causing uneven distribution of growth substance (▭: Mica plate. ➡: Direction of stipe splitting). A: Insertion after pre-treatment. B and D: Insertion to the untreated fruit body (top view).

C. Occurrence of the similar phenomenon in some other fungi.

When in *Coprinus radians*, *C. plicatilis*, *C. fimetarius* and *Psathyrella candolleana* a half of the pilei of young fruit bodies, in which no rapid elongation begins, were removed on the field, the stipes curved also to the pileus-free side as in *A. bisporus* (Photo. 3). But when the treatment is performed at later stages, at which rapid elongation of stipe has already begun, the curvature is not observed, perhaps because the sufficient amount of growth substance for the normal elongation has already been reserved (Borriss 1934)[2].

Discussion

It has taken a long time since Buller (1934)[1] and Borriss (1934)[2] to step the next progress, perhaps because of the booming studies of phytohormones in higher plants, before Urayama (1956)[3] stated that in *A. campestris* the gills supplies some growth stimulator to the stipe. In the present experiments, the authors have proved that the resulted curvature of the stipe is not caused by the cutting treatment itself and found that a certain growth substance occurring in the gills moves towards the stipe through the pileus-flesh or the partial veil.

The material mushrooms used in the present experiment are at about 30 mm. stage, at which the stipe seems to grow only by cell elongation. The growth stimulator produced in the gills and transported to the growth zone of the stipe acts upon these stages.

The fruit body withers sometimes when the gills are entirely removed, while it lives long so far as any gills are remaining. It is therefore obvious that the gills take an important part in the growth of fruit body.

The fruit body entirely deprived of the gills, however, continues to elongate

considerably after some grown stage, alike the cases observed by Magnus (1906)[6] on *A. campestris*, and Borriss (1934)[2] on *Coprinus lagopus*. This fact may show, as the latter author pointed out, that the growth stimulator might has already been reserved in the stipe tissue after it reached some stage.

Difference in the amount of gills remaining on each of the opposite sides also leads to stipe-bending. A proportional relationship to a certain extent between degree of curvature and amounts of gills, this is, amount of growth stimulator was observed. This fact suggests a possible method for bioassay of the stimulator for further investigation, especially in the case of extraction of this substance. The fundamental pre-treatment used in the present experiment is found to be a useful bioassay method.

From the phenomenon shown in Fig. 3 or from the experiments with mica plate, this substance seems easier to run downwards through the mycelial thread than to move laterally. From the fact that stipe curvature is caused by a small amount of gills, this substance seems to be active minute quantity.

The occurrence in gills, the promotion of pileus expansion and of stipe growth indicate that this stimulator is a "hormone" according to the definition of Went and Thimann (1937 .

The observations with fungi other than commercial mushroom, *viz. Coprinus* and *Psathyrella*, support the view that such a growth stimulator or hormone may extensively exist and play the same role in the growth of the fruit bodies in general.

Summary

1) Existence of a growth hormone active to stipe elongation of *Agaricus bisporus* was proved.

2) Removal of gills from one side of stipe, insertion of mica plate between pileus-flesh and gills caused curvature of stipe, showing close relation between the stipe elongation and existence of gills.

3) Growth of fruit body is controlled by the growth hormone produced in gills and transported down to growth zone of stipe through pileus-flesh and partial veil. The transportation seems to occur much easier in the longitudinal direction than in the lateral directions in the stipe.

4) Growth of fruit bodies of *Coprinus* and other fungi seems to be also regulated by the same principle.

5) A bioassay method of the growth hormone of fungi using fruit bodies of commercial mushroom as the tester was proposed.

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摘　　要

1. ツクリタケ（西洋マツタケ）で，子実体の生長促進ホルモンの存在を実験的に明らかにし，さらにかような生長ホルモンが，広く担子菌の子実体の生長に関与しているという考えを提示した。

2. 伸長生長の盛んな 30 mm. 位の子実体を用い，そのかさの前後の部分を除き，左右にのみかさをもつ植物体を実験に使用した（図 1)。この左右のうち，一方のかさからひだを完全に除くと，子実体の柄はひだの除去された側に屈曲する。またひだの残された側のかさに，いろいろの程度に傷をつけても屈曲が起る（写真 1)。ひだの量を，左右異つた程度に除くと，柄はひだの少なく残された側に屈曲する。さらに左右のかさのうち，一方のかさと，柄の接合点に雲母板を挿入すると，柄は雲母板の挿入された側に屈曲する（写真 2)。しかし一方のひだを除き，他方の残されたひだをもつかさと，柄の接合点に雲母板を挿入しても，柄は殆ど屈曲しない。

3. 子実体の柄の屈曲は，傷やこれに伴なう水分の発散によって起るのではなく，ひだの存在と密接な関係がある。

4. 生長ホルモンは，ひだでつくられ，かさおよび内皮膜を通って，柄の生長部に向基的に移動し，細胞の伸長を促進する。

5. ヒトヨタケ属，その他の担子菌の子実体の生長も，また同様な生長ホルモンにより調整されていることが，かさの一部除去により明らかとなった（写真 3)。

6. ツクリタケの子実体を用いると，いろいろの菌の子実体の生長ホルモンの検定が可能である。

Effect of Water Economy on Plant Growth I. Influence of Water Level Lowering on the Growth of Water-cultured Tobacco Plants*

by Tsumugu TOTSUKA** and Masami MONSI**

戸塚　績**・門司正三**：　タバコ水耕における水位低下と生長

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The relationship between plant growth and moisture conditions of soil has been tackled by many authors.[1] However, many of them were mainly concerned with xeromorphism or with the influence of drought on transpiration and water economy, and very few have been worked on the relationship of photosynthetic and respiratory activities to water economy of plants.[2,3,4]

Water deficiency and temporary wilting of leaves are often observed in many plant species in hot and bright afternoon of midsummer, even under the mesophytic and high humid conditions in Japan. But it has scarcely been studied analytically how the growth of plants is influenced by the water deficit which has temporarily been induced by unbalanced water economy.

In the present paper, it will be discussed how the growth of tobacco plants suffers from limitation of absorbing root surface caused by lowering the water level of water culture solution. For the elucidation of this, the water deficit, mineral nutrient supply, and photosynthetic and respiratory activity of the plant will be discussed respectively, and then the growth sequence will be pursued by calculating the dry matter production in connection with the leaf water deficit.

Material and Methods

As material, *Nicotiana tabacum* L. "Bright Yellow" was selected for the reason why it is capable of normal growth even in somewhat weak illumination of 5000 lux of artificial light to keep the growth condition as possible as constant, and why it is favorable to evaluate the amount of roots active in water absorption without disturbance of tap root system, as the latter develops rather poor in this plant.

Three weeks after germination, young plants selected in equal size were water-cultured with enameled pots to provide the material for the experiment. Out of these material plants three experimental plants per pot were selected, and trans-

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planted again to Wagner's pots (18 cm. in diameter, 18 cm. in depth) on April 14, 1958, the 6th day of water culture, when the root developed about 10 cm. in length. Great care was of course taken to avoid mechanical damages especially of roots when transplanting. The Wagner's pots were located in a growth cabinet under the conditions of continuous illumination of 4500-5100 lux (ten 40 watt fluorescent tubes, Mazda, white), at 25±0.5° (the reported optimum for tobacco plant growth was 23-29°[5]), relative humidity of 49-62%, or 12-9 mm. Hg in saturation deficit, and CO_2 concentration of 0.539-0.562 mg./l.

The water level of culture solution was maintained with some device at constant depths, 2, 4, 6 and 8 cm. (from the top of pot), throughout the course of experiments. The experimental plants were cultured lest they should shade themselves, and for each set 2 pots were prepared. Boysen Jensen's culture solution (pH=4.3) was used, being dropped in the pot through a fine plastic pipe to renew the solution by ca. 1 liter a day, in order to keep constant levels of nutrient and oxygen concentrations. All the values of the latter measured in each pot by Winkler's method (at 25°) fell near 7 mg. O_2/l.

Samplings of the material were done after 3, 7 and 11 days from the start of the constant water level treatment. At each sampling, fresh and dry weight of leaves, stems and roots, and leaf area were determined in two replications for each set.

Other two series of the same kind of experiments were carried out and have shown similar results.

Results and Discussion

1. The growth in dry weight:

The course of growth expressed in dry weight is shown in Table 1. The plants had the largest growth of 1755 mg. at the 2 cm.-set and the growth declined in parallel with the lowering of water level. The relative plant dry weights after 11 days of the experiment were 100, 87.5, 78.9 and 68.6 at the 2 cm.-, 4 cm.-, 6 cm.- and 8 cm.-sets, respectively, and this depression is severer than that in the leaf areas, where the relative values were 100, 93.2, 84.5 and 78.3.

For the sake of further analytical comparison of the growth difference, the net assimilation rate (NAR) of both extremes, the 2 cm.- and the 8 cm.-sets, was roughly calculated by the following equation (cf. e. g. Watson 1952[6]).

$$\mathrm{NAR} = \frac{\Delta W}{\bar{F}(t_2 - t_1)},$$

where ΔW indicates the increment of dry weight during the time interval of $t_2 - t_1$, and $\bar{F}$ is the leaf area at the time of t_1. The NAR expresses the efficiency of leaves in the growth rate. The NAR calculated are:

	April 14—17	17—21	21—25
at the 2 cm.-set	1.59	1.21	0.92
at the 8 cm.-set	1.22	0.87	0.83.

The values for the 2 cm.-set always obviously exceeded those for the 8 cm.-set.

As the decline of the NAR would be caused by the depression of net assimilation (=photosynthesis—respiration) of leaves, the increase of the ratio of non-photosynthetic organs to leaf area ($C/\overline{F}$ ratio) and the accerelation of respiration in non-photosynthetic organs (in this connection, see Iwaki 1958[7]), further discussions as to each of these factors will be done in the following.

The values of the $C/\overline{F}$ ratio were 0.97, 1.18, 1.01 and 1.24 at the 2 cm.-set, and 0.97, 1.19, 1.21 and 1.11 at the 8 cm.-set, on April 14, 17, 21 and 25, respectively. Little higher values were observed in the 8 cm.-set than in the 2 cm.-set. This is because the ratio of root fresh weight to the leaf area was rather larger at the 2 cm.-set than the 8 cm.-set, while in the ratio of stem dry weight to the leaf area there was almost no difference between the two sets. At any rate, no signficant difference in the $C/\overline{F}$ ratio was found out between the two sets. Accordingly it should be assumed that the difference in the NAR may be induced by the difference in photosynthesis per unit leaf area, and/or respiratory activities of non-photosynthetic organs.

Table 1. Effect of water level lowering (started April 14, 1958) on the growth in dry weight of each organ and in leaf area, and active root/leaf area ratio (fresh weight/sq. cm.).

Date	Water level lowering	Total dry weight		Leaf dry weight	Leaf Area	Stem dry weight	Root total	Root aerial	Root submerged	Active root /leaf area ratio
		mg.	rel.	mg.	sq. cm.	mg.	mg.	mg.	mg.	mg./sq. cm.
Apr. 14	2 cm.	170		116	55.7	27	27.0	12	15.0	4.38
	4 cm.	163		110	52.0	26	26.8	22	4.6	1.73
	6 cm.	166		113	54.6	26	27.4	25	2.4	0.72
	8 cm.	174		119	57.6	27	28.2	28	0.2	0.26
Apr. 17	2 cm.	436	100	316	102	46	74	46	28	4.4
	4 cm.	409	93.9	295	92	44	70	38	32	4.4
	6 cm.	381	87.5	272	88	42	67	34	33	3.9
	8 cm.	383	87.9	275	91	38	70	47	23	4.3
Apr. 21	2 cm.	929	100	706	223	102	121	53	68	5.1
	4 cm.	854	91.8	640	196	92	122	41	81	6.7
	6 cm.	798	86.0	592	181	77	129	70	59	5.7
	8 cm.	700	75.5	518	151	69	113	80	33	4.7
Apr. 25	2 cm.	1755	100	1314	357	201	240	135	105	4.8
	4 cm.	1537	87.5	1165	333	221	151	61	90	4.9
	6 cm.	1386	78.9	1050	302	171	165	91	74	4.6
	8 cm.	1204	68.6	893	280	131	180	107	73	5.0

The change of the total nitrogen content of leaves was investigated by the micro-kjerdahl method in order to clarify whether or no the reduction of active root surface below the normal can affect the absorption of mineral nutrients significantly. The results shown in Table 2 were the mean values of three determinations.

Table 2. Variation of total nitrogen content of the tobacco leaves (mg. N/g. D. W.)

Water level lowering	Apr. 14	Apr. 17	Apr. 21	Apr. 25
2 cm.	45.2	32.3	37.7	31.6
4 cm.	44.8	34.1	31.5	36.0
6 cm.	46.1	32.7	31.3	27.2
8 cm.	45.5	37.9	34.9	28.7

So slight difference in each set was observed, that it can hardly be suggested there was any deficiency of mineral nutrients in plant with limiting water absorption. Close relationships between photosynthetic activity and nitrogen content of leaves are well known[8),9),10)]. It does not seem, however, that the observed fluctuations in nitrogen content of the tobacco leaves may have any influence on the photosynthetic activity for 7 days after starting the experiment, when the difference clearly appeared in the growth.

2. The depression of the leaf water content:

The changes of water content of the individual organs were investigated after 24 hours from starting the water level lowering (see Table 3). All organs other than leaf blade showed no severe depression of water content on dry weight basis.

Table 3. Effect of water level lowering on the water content of leaf, stem, aerial root and submerged root (average values) after 24 hrs. of starting the experiment.

Water level lowering	Active root/leaf area ratio mg./sq. cm.	Water content (per cent on dry weight basis)			
		Leaf	Stem	Aerial root	Submerged root
2 cm.	4.5	907	1130	1203	1327
4 cm.	4.1	883	1250	1130	1293
6 cm.	1.8	865	1227	1190	1350
8 cm.	0.9	820	1218	1053	1375

The relation between water deficit of leaf blade and water level lowering is shown in Fig. 1. Here the water deficit is calculated on the leaf area basis (29 mg. H_2O/sq. cm. in saturated conditions of leaves) and the grade of water level lowering was expressed by the ratio of submerged root (mg. fresh weight) to leaf area (sq. cm.), i. e., active root/leaf area ratio, which may also indicate the water economy of various plant types to some extent. For further discussion as to these expressions another paper will be expected in the near future.

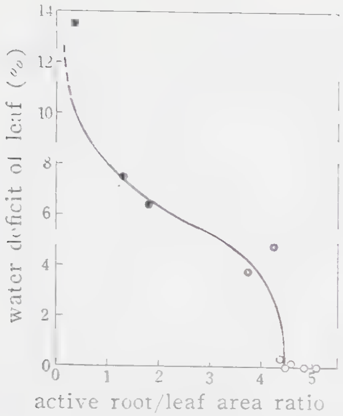

Fig. 1. Relationship between active root/leaf area ratio (mg. fresh weight/sq. cm.) and the water deficit of leaf (per cent on the leaf area basis) obtained from various water level lowerings (○ 2cm., ◎ 4cm., ◉ 6cm., ● 8cm.).

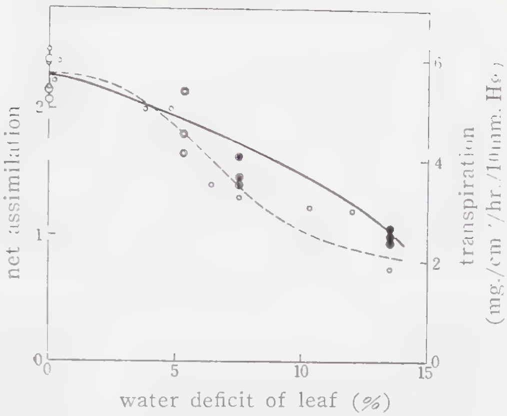

Fig. 2. Variation of net assimilation (continuous line) with the leaf water deficit caused by various water level lowerings (○ 2cm., ◎ 4cm., ◉ 6cm., ● 8cm.) and transpiration rate (broken line, small open circles).

3. Variations of net assimilation and respiration:

The depression of photosynthesis caused by the water level lowering was detected directly by a modified Boysen Jensen's method in intact leaves of tobacco plants water-cultured under the same conditions as in the experimental series. As clearly shown in Fig. 2, the values of net assimilation (mg. $C_6H_{10}O_5$/50 sq. cm./hr.) were decreased almost linearly until the leaf water content was decreased by 15 per cent below the normal. The respiratory activity of the leaf was hardly influenced by the leaf water deficit, its value was 0.49-0.54 mg. CO_2/50 sq. cm./hr. in each set. Therefore, the depression of net assimilation may not be expected from high respiration with water deficiency, but from the fact that the water deficit of leaves usually induces a closure of stomata, and accordingly a depression of photosynthesis as well as of transpiration, as already suggested by Watson[6] (see also Fig. 2). In this paper, however, the grade of stomatal opening was not especially taken into account, because the depression of plant growth caused by unbalanced water economy can be directly elucidated through the relation between net assimilation and water economy.

The reduction of water contents of roots and stems, as summerized in Table 3, was very small, so that it seems the variation of respiratory activity of these organs must be negligibly small and consequently need not to be taken into consideration regarding the depression of NAR.

From the above facts, it is evident that the decrease of the NAR at the 8 cm.-set resulted mainly from the depression of photosynthesis of leaves.

4. A theoretical calculation of the plant growth:

For the quantitative proof of the elucidation mentioned above, the causal relation of water level lowering to plant growth depression was pursued by calculating the dry matter production or the growth in connection with leaf water deficit. These calculations were carried out at the 2 cm.- and the 8 cm.-sets, for the first 7 days of the experiment before the plant recovering from the direct affection of water level lowering, and the techniques employed here were more or less the same as those used by Iwaki[7], excepting that the reduction of photosynthesis by leaf water deficit was the special concern in this paper.

The distribution ratio of the produced dry matter to the individual organs was calculated based on the data which were obtained at each sampling, provided this measure would vary linearly during sampling period. With assuming that the leaf and root grew logarithmically during each sampling interval, the variations with time of the active root/leaf area ratio were computed in Fig. 3.

The value of net assimilation of a plant under given conditions could be estimated by combining the data of Figs. 1, 2 and 3. Fig. 3 gives us the time sequence of the active root/leaf area ratio, with which the leaf water deficit can be read in Fig. 1, and with the values thus obtained the net assimilation will be easily computed from Fig. 2. At the 2 cm.-set, the active root/leaf area ratio varied so slightly that the value of net assimilation could be estimated as a constant value of 1.09 mg. $C_6H_{10}O_5$/sq. cm./day. At the 8 cm.-set, however, the net assimilation in each day dur-

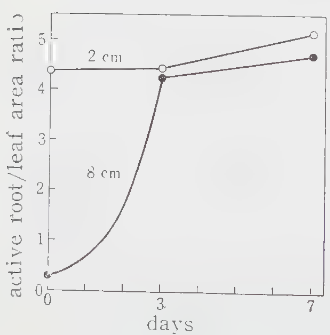

Fig. 3. Diagram showing the time trend of active root/leaf area ratio (mg./cm.²), calculated based on the growth of submerged roots in fresh weight and of leaf area.

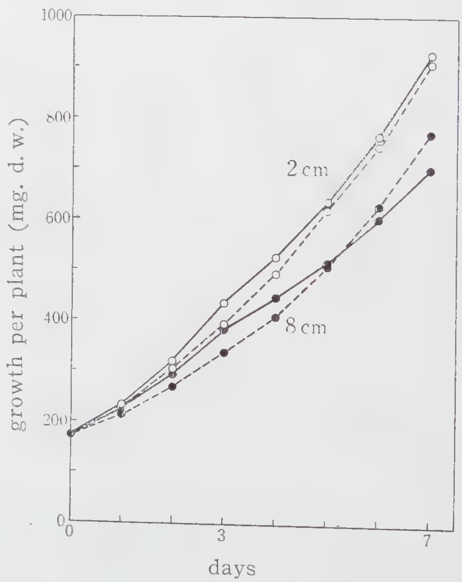

Fig. 4. Comparison between the observed growth (continuous line) and the calculated (broken line) at the 2 cm.- and the 8 cm.-sets.

ing 7 days from starting the experiment was determined as 0.75, 0.81, 0.89, 1.09, 1.09, 1.09 and 1.09 mg. $C_6H_{10}O_5$/sq. cm./day, respectively.

The respiration of stem was excluded from the calculation of growth, because it is very considerable that the stem bearing chlorophyll, as known in stems of raddish and pea,[11,12] can compensate its respiration with its own photosynthate in an illumination of 5000 lux. The respiration rate of the aerial and the submerged roots without water deficit was determined to be 0.096 and 0.146 mg. $C_6H_{10}O_5$/mg. dry weight/day, respectively, and there was no change in the water content, and consequently in respiration, with water level lowering, as already mentioned.

A fairly good coincidence between the observed and the calculated growth at the 2 cm.- and the 8 cm.-set is illustrated in Fig. 4, though the calculated is slightly lessened than the observed at any set—especially, at the 8 cm.-set, there are some deviations in the calculated, probably because of the fluctuation of sampling on April 17 and 21, and for the lower calculated value at the 2 cm.-set an underestimation of net assimilation under normal leaf water condition may be responsible. At any rate, from the fact that there is no extreme discrepancy between the observed and the calculated, it can be presumed that the data and methods which were empolyed for the calculation of the growth did not contain any fundamental errors.

In conclusion, the growth depression induced by the water level lowering or temporary unbalance of water economy results from the depression of photosynthesis caused by water deficiency in the plant, especially in leaves, during the period when the active root/leaf area ratio was recovering from serious decline after treatment up to its normal value, or the same value of the control.

Summary

1. The effect of water economy on the plant growth was investigated in young tobacco plants with water level lowering of culture solutions. The 2 cm.-, 4 cm.-, 6 cm.- and 8 cm.-sets were prepared.

2. The plant growth in dry weight was decreased in proportion to the water level lowering, i. e., the relative values of 100 : 87.5 : 78.9 : 68.6 were observed at the 2 cm.-, 4 cm.-, 6 cm.- and 8 cm.-sets, respectively. Also the lowest net assimilation rate (NAR) was obtained at the 8 cm.-set.

3. The ratio of the dry weight of non-photosynthetic organs to the leaf area indicated no difference among the sets.

4. With lowering the water level, heavy water deficit was induced in leaves, but no deficit was observed in stems and roots. A clear relationship was discovered between the leaf water deficit and the ratio of submerged roots to leaf area (active root/leaf area ratio), which was used here as an indicator of the grade of water level lowering.

5. The net assimilation decreased almost linearly with progress of the leaf

water deficit, while no change was observed in respiratory activity of leaves as to water deficit and no special depression of nitrogen content in leaves was detected during the experimental period.

6. The growth in dry weight at the 2 cm.- and 8 cm.-sets was calculated theoretically with the net assimilation, which was affected by the leaf water deficit caused by water level lowering. The agreement between the calculated and the observed proves that the primary cause of the growth depression with water level lowering is the suppressed photosynthesis of leaves.

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摘　　要

水分経済と物質生産の両面から，植物の一時的しおれがその後の生長にどのような影響を与えるかを追及するために，タバコ幼植物を水耕し，その水位を 2, 4, 6, 8 cm. の四段階に低下させ，約 5000 lux の連続照射，温度 25°，湿度 49～62%，CO_2 濃度約 0.55 mg./l. の条件のもとで栽培した。水位の低下につれて生長は減退した。すなわち実験開始 11 日後の乾物生長量の割合は 2, 4, 6, 8 cm. 区においてそれぞれ 100, 87.5, 78.9, 68.6 となり，最初の 3 日間の平均一日当りの純同化率 NAR は水位の低下の順に減少した。NAR を支配する各因子の変化は次のようであつた。a）非同化器官と葉面積との割合は各区において大差なかつた。b）水位低下により葉に水分欠乏が生じた。c）葉の水分欠差と，水位低下の度合を表わす根の生量に対する葉面積の割合（active root/leaf area ratio）との間には密接な関係があつた。d）葉の窒素含量には顕著な変化はみられなかつた。e）葉の水分欠差が進行するにつれて同化量はほぼ直線的に減少したが，呼吸量はほとんど変らなかつた。f）茎，根の含水量変化は認められなかつた。従つて茎，根の呼吸率の変化は起らなかつたと考えられる。

以上の諸事実にもとずいて生長の低下の原因をさらに確かめるために，葉の水分欠差と物質生産を結びつけて 2 cm. 区と 8 cm. 区の生長を計算した結果，実測値とほとんど一致した。それ故，水位低下によつて生じた生長量の減少は主として葉の水分欠乏による同化量の減少の結果といえる。

抄　　録

顕花植物のジベレリン I. インゲンのジベレリンの単離とその性質

West, C. A., and Phinney, B. O., Gibberellins from Flowering Plants. I. Isolation and Properties of a Gibberellin from *Phaseolus vulgaris* L. Jour. Amer. Chem. Soc. **81**: 2424 (1959)

ジベレリンは 1938 年藪田・住木によってイネの馬鹿苗病菌 (*Gibberella fujikuroi*) から粗結晶 [illegible] A_1, A_2, ジベレリン酸, ジベレリン B, C と多くの物質があり，高等植物にもおそらく含まれていて [illegible] る。著者らはすでにインゲンの種子の中に既知の [illegible] 質が存在することを報告している (1957)。この [illegible] 様物質 bean factor I, II を結晶状にとりだし，このうち bean factor II が今まで報告されたジ [illegible]

Phaseolus vulgaris L. の未熟種子約 25.2 kg. を [illegible] 1:1 で抽出し [illegible] ——酢酸エチル抽出——ケイ酸・ケイ藻土柱によ [illegible] トグラフィ (アセトンで展開)——向流分配法 (ブタノール：アンモニア緩衝液 pH 8) の諸操作を行なって，それぞれ約 2 mg. ずつの bean factor I および II を結晶として単離した。bean factor II は分解点 250~255° の小板状結晶である。活性 [illegible] 然変異種である矮性突然変異 dwarf 1 (d-1) および dwarf 5 (d-5) を用いている。

Bean factor I は菌類のジベレリンと同じように d-1, d-5 の生長を同じ程度に促進し，ジベレリン A_1 と多くの点で一致する。bean factor II は d-1 にはほとんど活性をもたないが，d-5 に強い活性をもつ点で特異的な物質である。

Bean factor II の赤外線吸収スペクトルはジベレリン酸と多くの類似点をもってはいるが，明らかにちがったものである。波数 1700~1800 cm^{-1} の吸収を比べると bean factor II の分子 [illegible] ているような カルボン酸と γ ラクトンがあるとみて矛盾はない。Rf 値は明らかにジベレリン酸，ジベレリン A_1, A_2 とちがっており，また硫酸溶液でジベレリン酸にみられる特徴のある青緑色螢光を示さない。このことはジベレリン酸の A 環に相当する環に，不飽和性のないことを示している。しかし，パラジウム・活性炭を触媒とする接触還元の結果ではジベレリン酸と同じ程度の不飽和性をもって [illegible] 紫外線吸収 [illegible] この不飽和結合が水酸基，ラクトンと共軛していないことを示している。

Bean factor II がトウモロコシの二つの突然変異種 d-1, d-5 に対してちがった生理的活性をもつことは，トウモロコシ内でのジベレリン生合成の径路に関係があると著者らは推論している。すなわち，ジベレリン酸やジベレリン A_1 のトウ [illegible] ように [illegible] することができ [illegible] 酸やジベレリン A_1 が正常のトウモロコシによってつくられたジベレリンとはおきかわりうるが，突然変異種に [illegible] でつくられた [illegible] とはおきかわりえないと仮定すると，bean factor II は d-5 の体内 [illegible] の体内では変らない中間体と考えられる。つまり，d-1 は bean factor II が活性ジベレリンに変る変化を遺伝的に [illegible] ていない変異体ということになる。

以上のように，単離した量がきわめて少ないので，はっきりとした諸性質は十分報告されていないが，[illegible] 前駆物質ともいうべきものを単離したことはかなり意味のあるものと思われる。なお著者らがその生物学的性質および生化学的，遺伝学的な関係の詳細を別に報告すると述べていることを附記する。（[illegible]関英雅）

植物の蛋白合成とその制御

Bonner, J., Protein Synthesis and the Control of Plant Processes. Am. J. Bot. **46**: 58-62 (1959)

植物成熟組織では多くはいわゆる "Endoplasmic reticulum" の膜様構造を欠き，遠心分画法によりいわゆる"ミクロゾーム粒子"だけがほぼ均質にわけとられる。これは長径 280 Å の扁楕円体で RNA 40%，蛋白質 60% からなり種類によらずおおよそ共通で，6 個のリボ核蛋白分子が Mg^{++} によつて可逆的に結びあわされてできている。標識アミノ酸を与えれば標識は速やかにミクロゾーム画分に現れる。しかし自身の標識はごく僅かでかつ一時的で無標識アミノ酸で容易に洗い落される。すなわち自身は"仲立ち"としての働らきをしているものである。トレーサー，DNase，除核等の結果からミクロゾームは核中で DNA の特異性に従って新生され，細胞質中へ転移することは明らかである。ミクロゾームは核内の DNA に含まれる潜在情報を伝達する"仲立ち"あるいは"遺産"であつて，いわゆる "template" 鋳型として働らき，それぞれの特異的活性アミノ酸を表面に集めてその固有の列に順序づけ蛋白質のペプチド鎖に作りあげるものと思われる。一個のミクロゾームに含まれ得るそのような適当な配列順序に関する情報には限りがあり，およそ数百のアミノ酸単位のために必要なものが含まれる。従って外見的には類似であっても実際にはそれぞれに互いに違つた暗号をもつもので個々のミクロゾームはそれぞれに特異な蛋白質合成に関係しているものである。結局ミクロゾームはいわば蛋白質合成のエンジンであつて，生長あるいは分化といつても結局は新らしい原形質の生産に基ずくものであるからこれらの過程に関する理解も窮極にはこのミクロゾームの特殊性に関する知見の進歩から導かれると思われ，ミクロゾームこそ現代生物学のもつとも基本的な重要問題の一つであろう。　(吉田 [illegible])

本　会　記　事

(近 畿 支 部)

6 月例会 (6 月 28 日，於奈良女子大)，総会：35 年度日本植物学会大会に関して。講演：[illegible]尚史：南紀地方の苔類フロラ。川戸峯子，信夫隆治：放線菌の一新種 *Streptomyces viridofaciens* について。植田勝巳：*Cyanidium* の微細構造。

(役 員 交 代)

7 月 1 日より，事情により編集幹事が一名交代いたしましたので，おしらせいたします。

旧幹事：[illegible]藤満彦

新幹事：黒岩澄雄

本会会員　平井一男氏は昭和 34 年 2 月 19 日に死去されました。
本会会員　野口つた氏は昭和 34 年 3 月 9 日に死去されました。

ここに報告し謹んで哀悼の意を表します。

日本植物学会

Physiological Studies on Photo-electric Response in Plant Tissues.
II. The Effects of Different Light Intensities on Photo-electric Response of Green Leaves.*

by Yuichiro NISHIZAKI**

[illegible]
II. [illegible]

Received March 6, 1959

In a previous paper[1], experimental results were reported on the effects of the duration of light and dark periods before illumination on the photo-electric response in green leaves. It has been shown that the photo-electric response was affected by the duration of the dark period before illumination, and was considerably suppressed by prolonged pretreatment in the dark.

In this paper, the changes in photo-electric response were investigated at different light intensities in relation to the pre-illumination on the leaves.

Material and Method

Green leaves used for the experiments were obtained from the seedling of *Phaseolus vulgaris* L. which had been cultured in a greenhouse for about three or four weeks. The leaflets of the second or third leaf with petiolules were separated from the petiole and immediately immersed in tap water to be kept during the experiments.

The experimental arrangements and the apparatus used were essentially the same as described in the previous paper[1], except for some partial improvements. In the experiments, a leaflet was covered with black paper having a circular hole 2 cm. in diameter through which a part of the leaflet was illuminated. Non-polarizing electrodes of $Zn/ZnSO_4$ were employed in the measurement of the leaf potential. These were connected through KCl-agar bridges with the starch paste closely in contact with the lower surface of the leaflet at two definite positions, the one under the center of the hole in the black paper and the other under the covered portion.

For the illumination, incandescent light from a projection lamp (100 V, 200W) equipped with a lens system was used. Heat was removed by inserting a filter of a 5 cm.-thick layer of a $CuSO_4$ solution (6%). By changing the distance between lamp and object on an optical bench, the intensity of the light could be adjusted

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within wide limits. The intensity of the illumination was measured by means of an illuminometer (TOSHIBA, No. 5). The temperature during the experiments was 14–15°.

Experimental

A. *Photo-electric response under illumination of different intensities.*

A series of experiments was performed to observe the relation between the photo-electric response and the light intensity. It has been shown in previous papers[1,2] that the magnitude and the course of the photo-electric response are affected by the length of the dark period before illumination. Furthermore, a pre-illumination preceding a relatively short dark period also changes the ensuing light reactions[1] (see below).

Therefore, experiments were made as follows: the exposed part of the leaflet was pre-illuminated with the given light intensities for 30 minutes to obtain a full response by the test-illumination for 10 minutes at the same intensities after the interruption of five minute darkness. The response produced by these test-illuminations was compared with reference to the light intensities of 1,000, 2,000, 4,000, 8,000, 16,000 and 32,000 lux, respectively.

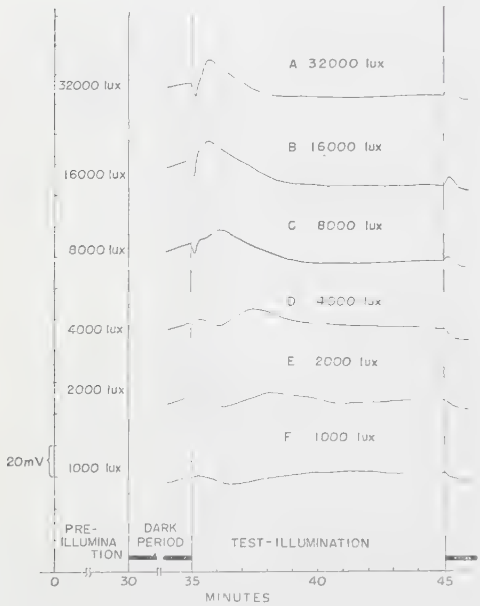

Fig. 1. Photo-electric response under different light intensities. Prior to the test-illumination, the leaves were subjected to a pre-illumination of 30 minutes at each light intensity followed by a dark period of five minutes. The upward changes of the curve show that the illuminated portion of the leaflet is negatively charged as compared with the unilluminated portion. The dark period is indicated by the heavy line on the abscissa.

The results are shown in Fig. 1. When the light of 1,000 lux was applied, insignificant variations in the electromotive force were elicited between the illuminated and unilluminated portions. With the light intensity increased to 2,000 lux, two slow negative (upward) peaks were clearly demonstrated. The new small "cusps"[1,3], which first carry the potential difference in the positive (downward) direction before it rises, appeared at light-intensities of 4,000 lux and above. As the light-intensity is increased, the two ensuing negative peaks become increasingly higher and finally fuse into an apparently single peak.

On close investigation of the transition of the negative deflections in the curves in Fig. 1, it is evident that there are essentially two separate components in the negative phase. On illumination at lower light-intensities these two negative components can be distinguished by the difference in time required to reach the respec-

tive peak maxima. When an intense light is applied, however, the maximum of the second peak gradually approaches the first one and finally overlaps it.

B. *Effects of the pre-illumination on the response.*

In the above experiments, the same intensities of light were used in each set of pre- and test-illuminations. Therefore, the amounts of radiation that the leaves received during the pre-illumination varied with different light intensities. The results of the experiments in which different intensities of test-light were applied after pre-illuminating for 30 minutes with light of the 32,000 lux (followed by a dark period of five min.) are shown in Fig. 2 A, B and C.

Both Curve B in Fig. 2 and Curve C in Fig. 1 represent the responses produced by the same test-light of 8,000 lux. It is clear from these curves that the response in the former case, which received a greater amount (or intensity) of radiation in the pre-illumination, is greater than in the latter, which received a lesser amounts (or intensity). The former has an obviously large negative peak while the latter shows two separate peaks.

It is evident from this fact that the photo-electric response increases when the amount (or intensity) of pre-illumination is increased. The same conclusion was derived by comparing Curve C in Fig. 2 with Curve F in Fig. 1.

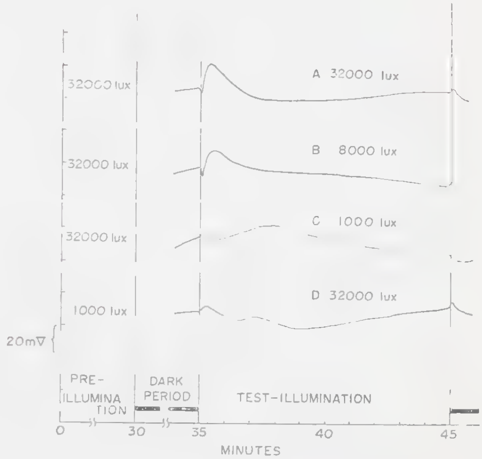

Fig. 2. Effects of pre-illumination on the photo-electric responses. Prior to the test-illumination, the leaves were kept in darkness for five minutes after the pre-illumination of 32,000 lux for 30 minutes in A, B and C, and of 1,000 lux for 30 minutes in D (Cf., Fig. 1).

On the other hand, a typical example of the effect of decrease in pre-illumination on the potential change in intense test-light (32,000 lux) is shown in Curve D as compared with Curve A in Fig. 2. The electrical response in Curve D with 1,000 lux pre-illumination was found to be less prominent as compared with that in Curve A with 32,000 lux pre-illumination; not only the magnitude of the response was decreased but also the second peak appeared as was the case in experiments with lower test-illumination intensity (1,000 lux; Curve C, Fig. 2).

The record of the photo-electric response during a period of intermittent illumination (five minutes light—30,000 lux—and five minutes dark) following a dark period of 30 minutes is shown in Fig. 3. It is clearly shown in this figure that the light response which was insignificant in the first light period became more enhanced on intermittently repeated illumination. At the same time, the two peaks, which appeared in the response curves of the first three periods, fused into a large single peak during the final illumination period.

It is concluded from the above experiments that the light-induced electrical

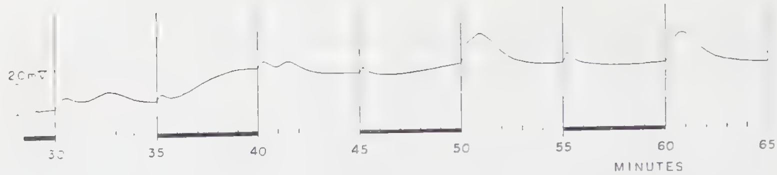

Fig. 3. Photo-electric response by intermittent illumination (five minutes light—30,000 lux—and five minutes dark) following a dark period of 30 minutes. The dark period is indicated by the heavy line on the abscissa.

response of the leaves is increased within certain limits by increasing the light intensity of pre-illumination and/or the intensity of the test-light.

C. *Effects of varied pre-illumination with a fixed amount of energy.*

Further experiments were made to ascertain whether the light-induced photo-electric response was the same with pre-illuminations with a given energy, but applied at different light-intensities and for different exposure periods. The test-light of 10,000 lux was applied after varied pre-treatment at a constant energy followed by a dark period of five minutes. The results of the five experiments of this series are shown in Fig. 4, in which pre-illumination of 2,000 lux-25 min., 5,000 lux-10 min., 10,000 lux-five min., 20,000 lux-2.5 min. and 50,000 lux-one min. were applied, respectively. It is clear, judging from the comparison of the electrical responses induced by a fixed test-light of 10,000 lux, that the reciprocity law does not hold in so far as the effects of the pre-illumination are concerned. The pre-illumination of 10,000 lux-five min. (Curve C) induced the greatest response to the test-light. As either the power or period of pre-illumination decreased, the corresponding response decreased.

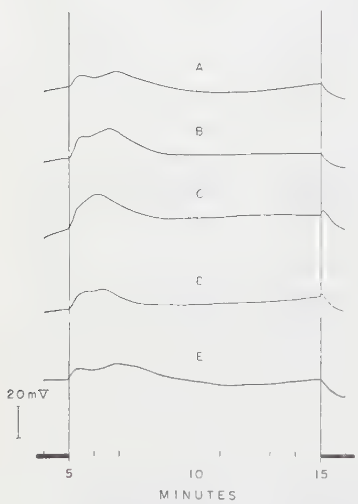

Fig. 4. Effects of pre-illumination with a fixed amount of energy on the photo-electric responses. The leaves were pre-illuminated with the light of 2,000 lux-25 min. (A), 5,000 lux-10 min. (B), 10,000 lux-five min. (C), 20,000 lux-2.5 min. (D), and 50,000 lux-one min. (E), and thereafter kept in the dark for five minutes, prior to the test-illumination at 10,000 lux.

Discussion

The results obtained from the above experiments have an interesting bearing on the nature of the photo-electric response. It is shown that the photo-electric response consists essentially of two separate components, each with a negative potential defined above. At high light intensities these two components overlap one another and appear as a single peak.

There are two factors to be considered as regards the cause of the light-induced

response under investigation. The one is the different effects of the light in different regions of the spectrum and the other is the reaction supposed to take place in the leaf after the absorption of the light. There is a possibility of obtaining a two-peaked response if the leaf were equipped with two separate systems, each absorbing light of different wave lengths and reacting in a separate way, and with a different time of response. An alternative explanation would be to assume that there are two response systems with different reaction time, but with an identical (or common) action spectrum.

For the purpose of obtaining material basis to decide this point, a comparison was made between the effect of a monochromatic light and that of the white light in test-illumination. It was revealed that a monochromatic red light obtained by passing through an interference filter (λ_{max} : 665 mμ, T_{max} : 44.0%, $\Delta\lambda^1/_2$: 14 mμ) gives rise to a photo-electric response with two peaks quite similar to that evoked by the white light at certain level of intensity. This fact indicates that the occurence of two peaks is not attributable to the participation of two separate mechanisms with different action spectra, but supports the view that they are most probably caused by the difference in reaction time of the two mechanisms assumed to take place in the leaf after the absorption of the light. The physiological changes underlying the electrical response, however, still remain unelucidated.

It was revealed from the above-described results that the first negative phase of the response (i.e., rise in the potential curve) appears immediately after the illumination is begun. It was also noticed in some cases that a small "cusp" (i.e., drop in the potential curve) preceded the first negative phase of the response. The appearance of this "cusp" is most reasonably correlated with higher intensities of test-light, no such "cusp" being observed at light intensities lower than 2,000 lux (Cf., Figs. 1 and 2). It will also be noticed in these figures that the runs with a cusp at the beginning of light-period are marked by a transitory appearance of a small peak on turning off the illumination. The time, t, that elapsed before the second (main) peak in the potential curve appeared, on the other hand, was found to become more and more shortened as the light-intensity was increased, the two peaks finally being united into a single peak. The magnitude of the second peak is also a function of light-intensity, a high level being attained with stronger illumination. What immediately follows from these circumstances is that with higher light-intensities a potential curve is obtained which is characterized by the appearance of a small "cusp" at the commencement of the light-period followed by a main peak leading finally to a stationary level of potential (see Curve A, in Fig. 1).

The effect of light-intensity on the reaction time t (i.e., the time that elapses before the second, main peak) is illustrated in Fig. 5. The values in this figure are taken from the experimental results shown in Fig. 1. Similar experiments always give rise to approximately the same curve; a rectangular hyperbola. Tröndle[4] has investigated the effects of light on the permeability of protoplasm and found that the time that elapsed before the change in permeability occurred (i.e., reaction time) was a function of light intensity, and that the relation between the reaction time and the light intensity was given by a rectangular hyperbola. The reaction

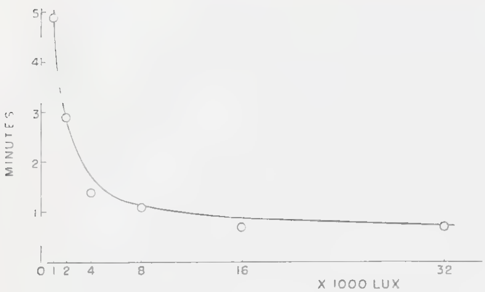

Fig. 5. Reaction time (t) for the second peak of response as a function of intensity of illumination (circles; taken from Fig. 1 and the theoretical curve; calculated from Equation 1).

time t (min.) in the present case can be calculated according to his equation[4)]

$$t=\frac{5+(i-1)\,0.6}{i}, \tag{1}$$

or

$$i(t-0.6)=4.4 \tag{2}$$

where i is the intensity of the light in units of 1,000 lux.

This formula means that the product of the light intensity and the reaction time minus a constant (0.6 min. in the equation) is constant so far as the experimental conditions in Fig. 1 are concerned. In this case, the reaction time, t, minus 0.6 min. may correspond to the presentation time (Präsentationszeit, Tröndle). Therefore, the above formula represents that the product of the light intensity and the presentation time is constant. It may be inferred from this fact that, in the photo-electric response under investigation, a certain process which presumably obeys the constant stimulus quantity-law is involved in a light receptor system in the leaf, and is accompanied by the potential change after the lapse of constant time (0.6 min.), independent of the light intensity.

The above-described results indicate that the intensities of the pre-illumination have a significant influence on the subsequent photo-electric responses and that the pre-illumination with intensity of radiation at constant energy have some different effects on the subsequent photo-electric responses caused by the same intensity of test-light. From these facts, it may be suggested that the photo-electric response in question is a really complicated reaction, in which certain secondary effects of some unstable intermediary substances formed as a result of pre-illumination may also be involved. Further investigations should be performed to make clear the actual mechanism of the events involved.

Summary

1. The changes in the photo-electric response in the leaves of *Phaseolus vulgaris* were investigated under different light-intensities and in relation to the pre-illumination.

2. The photo-electric responses of this material are essentially composed of two separate components in the negative phase as clearly shown in low light-intensities.

3. The response is increased by the pre-illumination of high intensity and is decreased by that of low intensity within certain limits.

4. The pre-illumination at a constant energy, but at varied intensities, of radiation does not result in an identical effect in response; i.e., the reciprocity-law does not hold in the pre-illumination.

5. A certain process which presumably obeys an extended form of the constant

stimulus quantity-law is inferred to be involved in the reaction underlying the second negative phase of the response.

The author wishes to express his cordial thanks to Dr. Y. Oda for his advice and encouragement throughout this work.

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1) Nishizaki, Y., Sci. Rep. RITU. D **9**: 107 (1958). 2) ———, Kagaku (Science) **27**: 464 (1957) (in Japanese). 3) Blinks, L. R., Jour. Gen. Physiol. **23**: 495 (1939). 4) Tröndle, A., Jb. wiss. Bot. **48**: 171 (1910).

摘　　　要

1. *Phaseolus vulgaris* の葉を用い，種々な強さの光を照射した場合に起こる光電反応の変化を，前照射の影響をも含めて研究した。

2. 光電反応は，強い光では1つの成分からなり，光が弱くなると反応は小さくなると同時に2つの成分に分離する。[illegible]は2つの成分が重なって現れたものであることが分る。

3. 強い前照射によって光電反応は大きくなり，逆に弱い前照射によって反応は小さくなる。

4. [illegible]にならず，前照射の強さ，照射時間の変化に従って増大あるいは減少がみられる。すなわち前照射に関しては，reciprocity-law が成立しない。

5. 光電反応の第2の成分の peak があらわれるまでの時間と光の強さとの関係は1つの直角双曲線で示され，この反応には刺戟量法測に従がうようなある過程が含まれていることが推論される。

Investigation with the Mäule Reaction on Plant Tissues I.

by Kozo NISHIO*

西尾康三*: 植物組織に対するモイレ反応の研究

Received March 18, 1959

Introduction

It is a general rule that angiosperm woods give the red color with the Mäule test for lignin, while gymnosperm woods give the yellow-brown color with the test[1),2)]. On oxidation with nitrobenzene, only vanillin was obtained from gymnosperm woods, while a mixture of vanillin and syringaldehyde in various ratios, typically 1 : 3, was obtained from angiosperm woods[3),4)]. It has been accepted that the red color is attributed to the syringyl group in lignin and the yellow or yellow-brown color is attributed to the guaiacyl group in it[5),6)]. However, precise studies showed us that there is a continuous series of plants between typical angiosperms and typical gymnosperms[2),3),4)].

By a histochemical investigation on the plants which are intermediate, we would be able to find plants which possess Mäule positive cells at the same time and to make a good contribution to the study on the physiology of lignification. However, we find few works in this field except Gibbs[4)], who gave some histochemical information in his extensive review on Mäule reaction and the oxidative products of various kinds of woods.

The present work applied the Mäule test to those plants which would be supposed to be the intermediate type in lignification. Samples to be observed were in the living state prepared by making hand sections. The results of the Mäule test were observed on the cells which had been known as lignified ones by the reaction with phloroglucinol and hydrochloric acid on as similar section as possible.

Result and Discussion

Species of *Podocarpus* showed much inconsistency with the Mäule reaction, and this occurred even in samples from different sources of the same species in this genus[2),3),4)]. In analytical data the species of this genus showed various ranges of rates in two kinds of aldehyde. In the present work the author observed that in *Podocarpus macrophylla* the cells with positive reaction (red color) and those with negative reaction (yellow or yellow-brown color) were found mixed one with another in the phloem as well as in the xylem. Thus, in xylem, the layers of positive colored cells were located alternatively with those of negative colored cells, and showed in stripes (Fig. 1 and 2). The ratio of two kinds of cells in sections varied

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considerably among the samples from different plants and even among those from different parts of the same plant. The sclerenchyma cells in the subepidermal region[4] and the pith showed positive color even when negative cells were dominant in the xylem part.

In angiosperms which possess both Mäule positive cells and Mäule negative cells, Gibbs[4] pointed out that more positive reaction was designated in the bundle sheath and the fibers than in the xylem. However, the author's observation on *Ephedra distachia* and some angiosperms showed more positive color in the xylem cells than in the phloem fibers (*Smilax Sieboldii*, *Kalopanax innovans*, *Acanthopanax sciadophylloides*, *Ephedra distachia*). The fact found by Gibbs[4] that the protoxylem often has a tendency to show negative color in Mäule reaction was corroborated by the author in his previous work[7] (*Chenopodium album*) and in his present work (*Kalopanax innovans*, *Ephedra distachia*). Creighton[3] got a high ratio of vanillin to syringaldehyde (1 : 0.8) on the oxidation with nitrobenzene. In the author's observation some samples of *Phyllostachys bambusoides* gave negative color in every cell, while the other samples of this species showed negative color in the sclerenchyma cells and in the bundle sheath of vascular bundles, located in the periphery region (Fig. 6).

In the xylem of *Illicium anisatum* the author found an interesting fact that both positive color and negative color with the Mäule reaction were provided in the same cell as well as in the same tissue (Fig. 3, 4 and 5). This tells us that two different biochemical situations, for producing the lignin of coniferyl type and that of syringyl type can exist at the same time not only in the same tissue but in the same cell.

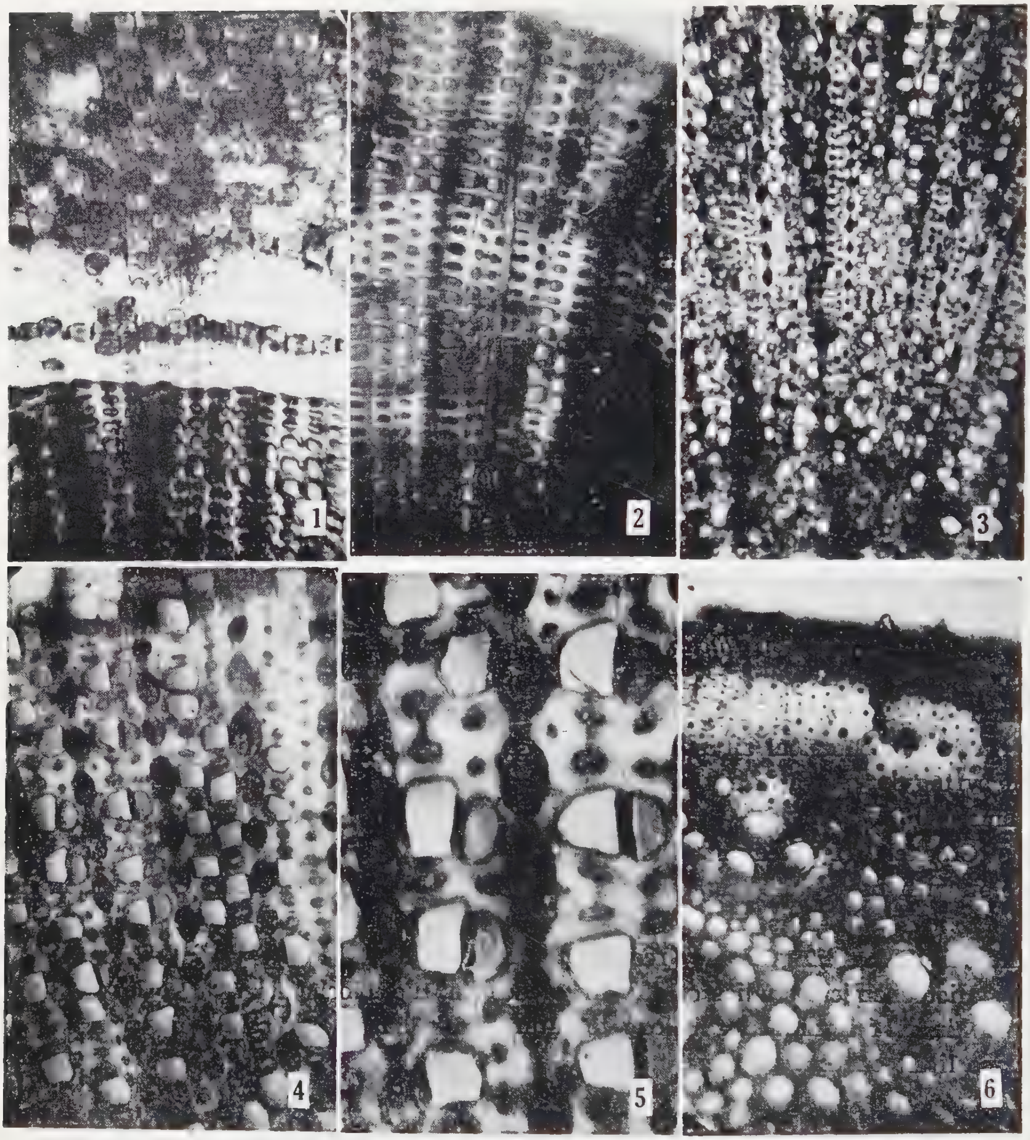
Crocker[2] reported that thorns of some angiosperms failed entirely to give red color with the Mäule test. The author's observation showed that in the thorn of *Smilax Sieboldii* cells in the periphery region indicated more negative color and the color changed gradually reddish toward the center (Fig. 6).

Histochemical studies on the difference in the biochemical situation between two kinds of lignification belong to future work, but the present work will throw some light on that problem.

Summary

The Mäule test was applied to those plants which would be supposed to be intermediate between the coniferyl type and syringyl type in lignification. Tissues of those plants very often showed various grades in color from red to yellow in the same plant. In *Podocarpus macrophylla* this occurred in the xylem, showing stripes of red and yellow. In *Illicium anisatum*, furthermore, both positive and negative colors with the Mäule reaction were provided in the same cell as well as in the same tissue.

The author is grateful to Takeda Herbal Graden in Kyoto which offered many good plant samples for his present work.



Mäule reaction on plant tissues.

Fig. 1. Phloem of *Podocarpus macrophylla*, ×200.
Fig. 2. Xylem of *Podocarpus macrophylla*, ×200.
Fig. 3. Xylem of *Illicium anisatum*, ×100.
Fig. 4. Xylem of *Illicium anisatum*, ×200.
Fig. 5. Xylem of *Illicium anisatum*, ×460.
Fig. 6. Young branch of *Phyllostachys bambusoides*, ×200.

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摘　　　要

リグニン化における，コニフエリル型とシリンギル型の両者の中間に位すると思われる植物について，モイレ反応を検した。これらの植物は，しばしば赤色から黄色にいたる種々の程度の色合を呈した。*Podocarpus macrophylla* の木質部では，赤色と橙黄色の細胞が縞をなして存在し，*Illicium anisatum* においては，木質 [illegible]

Studies on the Light Controlling Flower Initiation of *Pharbitis Nil.**

III. Light-sensitivity of the First Process of Inductive Dark Period

by Atsushi TAKIMOTO** and Katsuhiko IKEDA**

滝本 敦・池田勝彦： アサガオの花芽形成を支配する光条件について
III. 暗期反応初期段階の光感受性

Received March 25, 1959

As had been shown in previous papers[1)2)], when the dark period was preceded by a 4-hour illumination with white light of 10 lux or far-red light, the critical length of the dark period for the induction of flower initiation was shortened by some 4 hours in *Pharbitis* seedlings. It was suggested that one part of the process taking place in the first hours of the dark period can proceed under these lights. In the present investigation, this relatively light-insensitive partial process included in the inductive processes which take place in the dark period was examined in detail.

Material and Methods

The material used was the seedling of *Pharbitis Nil*, strain "Violet". Procedure of experimentation, light filters and light sources were similar to those described in the previous paper[3)]. Energy distribution of the spectrum of the light used was also shown in the same paper[3)].

Experiments and Results

Experiment 1. When 4 hours of illumination by the daylight fluorescent light of 10 lux (ca. 50 erg/cm^2./sec.) precedes the dark period, the critical dark period is shortened by some 4 hours, as had been indicated in the preliminary report[1)]. In the present experiment, 4 hours of illumination by daylight fluorescent light of 50 erg/cm^2./sec. mixed with far-red light (700～1000 mμ) of 120 erg/cm^2./sec. (FL+FR) were given prior to dark periods of various lengths, and the effect of this low-

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* The previous paper in this series was incorrectly titled, "Studies on the light controlling photoperiodic induction of *Pharbitis Nil.* II.", Bot. Mag. Tokyo, 72: 181 (1959). The title should have been "Studies on the light controlling flower initiation of *Pharbitis Nil.* II".

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intensity illumination upon flowering responses was examined. Results are shown in Table 1.

Table 1. Flowering response of plants exposed to dark periods of various lengths preceded by 4-hour FL+FR illumination.
FL+FR: Daylight fluorescent light of 50 erg/cm.^{2}/sec. mixed with far-red light of 120 erg/cm.^{2}/sec.
(Treated on May 15 and dissected on June 2, 1958)

Light preceding dark period	Length of dark period in hours	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
4-hour FL+FR	12	40	60.0	0.7	0
	10	39	87.2	1.1	0
	9	39	71.8	0.7	0
	8	40	10.0	0.1	0
	6	39	0	0	0
4-hour white light of 4000 lux (Cont. 1)	12	38	44.7	0.5	0
	10	40	0	0	0
	9	37	0	0	0
	8	40	0	0	0
	6	39	0	0	0
Sun light (Cont. 2)	16	40*	100	4.0	57.5
	14	40*	100	4.6	77.5
	12	40*	100	3.2	10.0
	10	40*	10.0	0.1	0

* The plants were kept in the dark from the beginning of the pre-illumination of other lots.

Plants subjected to FL+FR for 4 hours initiated flower primordia when the FL+FR illumination was followed by the dark period of 8 hours or more, but the control plants subjected to white light of 4000 lux (daylight fluorescent light mixed with incandescent light) for 4 hours did so only when a 12-hour dark period followed (Control 1, in Table 1). The critical length of the dark period is thus reduced by 4 hours compared with the control plants. This suggests that the first process of the inductive dark period can, also, proceed under FL+FR.

Flowering responses of the plants subjected to FL+FR for 4 hours and subsequently to an 8- to 12-hour dark period were, however, weaker than those of the plants given a 12- to 16-hour dark period preceded by bright sun light (Control 2, in Table 1). As was reported in the preliminary paper[1], the plants exposed to daylight fluorescent light of 10 lux (FL), which contained a small amount of far-red light, for 4 hours and subsequently to an 8- to 12-hour dark period initiated flower primordia to the same extent as those subjected to 12∼16 hours of darkness preceded by sun light. The flower inhibitory effect of 4-hour FL+FR is perhaps attributable to the action of the far-red light included in it. The far-red light is considered to inhibit the process taking place in the second or the later phase of

the dark period, but not that in the first phase, as has been suggested in the previous paper[2]. Further data supporting this hypothesis will be given in Experiments 3, 4 and 7.

In this first experiment the plants which were subjected to a dark period preceded by sun light initiated more flower primordia than those preceded by 4 hours of white light of 4000 lux, indicating that the latter is not strong enough to bring about the maximum flowering response when followed by the inductive dark period.

Experiment 2. Plants were exposed to the illumination by daylight fluorescent light of 50 erg/cm².⁄sec. for 8 hours, and subsequently kept in darkness for various periods, ranging from 6 to 16 hours. Control plants were exposed to 6 to 16 hours of darkness preceded by sun light. Results are shown in Table 2. A 6-hour dark period can induce flowering when preceded by 8 hours of illumination by daylight fluorescent light of 50 erg/cm².⁄sec. The control plants initiated flower primordia only when the dark period was 12 hours or more. The critical length of the dark period is thus shortened by 6∼8 hours in this case.

Table 2. Flowering responses of *Pharbitis* seedlings induced by dark periods of various lengths preceded by 8 hour illumination by daylight fluorescent light of 50 erg/cm.²/sec. (Treated on May 26 and dissected on June 8, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
8^hFL→16^hd	39	100	4.5	94.9
,, →14^hd	39	100	4.9	100
,, →12^hd	40	100	3.9	67.5
,, →10^hd	39	100	4.4	74.4
,, →8^hd	40	100	2.6	17.5
,, →6^hd	39	61.6	0.7	0
16^hd	39	100	4.3	100
14^hd	39	100	1.9	2.6
12^hd	39	5.1	0.1	0
10^hd	40	0	0	0
8^hd	40	0	0	0
6^hd	40	0	0	0

8^hFL→16^hd: 8-hour daylight fluorescent light of 50 erg/cm.²/sec. followed by 16 hours of darkness.

These notations will be used hereafter.

Experiment 3. Plants were exposed to FL+FR for 8 hours, and subsequently kept 6 to 16 hours in the dark. Control plants were exposed to 6 to 16 hours of darkness preceded by sun light. Results are shown in Table 3.

Eight-hour FL+FR inhibited flowering when followed by a 14- to 16-hour dark period, but promoted flowering when followed by an 8- to 10-hour dark period. This may be interpreted by the following assumptions.

Table 3. Effect of 8-hour FL+FR illumination preceding dark periods of various lengths upon flower initiation of *Pharbitis* seedlings
FL+FR: Daylight fluorescent light of 50 erg/cm.²/sec. mixed with far-red light of 120 erg/cm.²/sec.
(Treated on May 27 and dissected on June 11, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
8^hFL+FR→16^hd	39	61.5	0.6	0
〃 →14^hd	40	70.0	0.8	0
〃 →12^hd	38	36.9	0.4	0
〃 →10^hd	40	37.5	0.4	0
〃 → 8^hd	40	42.5	0.5	0
〃 → 6^hd	36	0	0	0
16^hd	43	100	4.3	100
14^hd	40	100	1.9	2.5
12^hd	40	32.5	0.3	0
10^hd	40	0	0	0
8^hd	39	0	0	0
6^hd	39	0	0	0

During the 8-hour FL+FR, the first process of the inductive dark period proceeds, but at the same time, the flower inhibitory effect of the far-red light takes place[2]. The dark period of 14~16 hours is effective to the maximum extent for floral initiation, and a dark period longer than 16 hours hardly increase the response. Therefore, when the FL+FR precedes a dark period longer than 16 hours, the flower inhibitory effect alone appears. On the other hand, an 8- to 10-hour dark period induces little flowering but further lengthening of the dark period promotes flowering response remarkably, and in such a case the promoting effect of the FL+FR preceding the dark period exceeds the inhibitory one, resulting in flower promotion. The critical dark period was thus shortened by some 4 hours.

Experiment 4. Plants were exposed to the far-red light of 100 erg/cm²./sec. for 4 or 8 hours and subsequently to a 6- to 16-hour dark period. Results shown in Table 4 are similar to those of Experiments 1 and 3, in which 4- or 8-hour FL+FR was given prior to a 6- to 16-hour dark period.

It is interesting that, when an 8- to 16-hour dark period was preceded by 8 hours of far-red light, flowering response did not vary with the length of the dark period. The same was also the case in Experiment 3, in which the plants were subjected to an 8- to 16-hour dark period preceded by 8-hour FL+FR. This suggests that the far-red light acts not to prevent flowering response at lower level, but to reduce the maximum level of the response.

Experiment 5. In this experiment, plants were exposed to daylight fluorescent light of 50 erg/cm²./sec. for 0, 2, 4, 6 or 8 hours and subsequently kept in darkness for 16, 14, 12, 10 and 8 hours, respectively, so as to make the sum of the length of the weak light illumination and that of the dark period 16 hours in each lot. Con-

Table 4. Effect of 4- and 8-hour far-red light of 200 erg/cm.²/sec. preceding dark periods of various lengths upon flower initiation of *Pharbitis* seedlings.
FR: Far-red light of 200 erg/cm.²/sec.
(Treated on July 14 and dissected on July 24, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
4^hFR → 16^hd	40	100	2.6	35.0
,, → 12^hd	37	100	3.0	35.1
,, → 10^hd	38	100	1.9	7.9
,, → 8^hd	39	61.5	0.6	0
,, → 6^hd	39	0	0	0
8^hFR → 16^hd	39	12.8	0.1	0
,, → 12^hd	38	18.4	0.2	0
,, → 10^hd	40	20.0	0.2	0
,, → 8^hd	40	27.5	0.3	0
,, → 6^hd	40	2.5	0.0	0
24^hd	37	100	4.7	89.2
20^hd	39	100	4.3	92.3
18^hd	40	100	4.2	97.5
16^hd	40	100	4.1	97.5
14^hd	38	100	4.1	92.1
12^hd	39	97.4	1.6	2.6
10^hd	40	0	0	0
8^hd	40	0	0	0

Table 5. Flowering responses of *Pharbitis* seedlings exposed to 16 hours of darkness, during the first period of which daylight fluorescent light of 50 erg/cm.²/sec. was given for various hours.
(Treated on May 15 and dissected on June 2, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
2^hFL → 14^hd	31	100	4.4	67.8
4^hFL → 12^hd	38	100	4.0	60.5
6^hFL → 10^hd	39	100	3.4	33.3
8^hFL → 8^hd	37	89.2	1.8	0
16^hd	34	100	3.7	44.1
14^hd	39	97.4	2.7	2.5
12^hd	39	5.1	0.1	0
10^hd	38	0	0	0
8^hd	40	0	0	0

trol plants were exposed to 16-, 14-, 12-, 10- and 8-hour dark period preceded by high-intensity light (4000 lux). Results are shown in Table 5. The plants exposed to the daylight fluorescent light of 50 erg/cm²./sec. for 2, 4 or 6 hours followed by

14, 12 or 10 hours of dark period respectively initiated flower primordia to the same extent as those exposed to a 16-hour dark period preceded by high-intensity light. This implies that the process which proceeds during the first 6 hours of the dark period can proceed under this low-intensity light with the same ease as in the darkness.

Experiment 6. Plants were exposed to daylight fluorescent light of various intensities for 6 hours and subsequently to 10 hours of darkness. Flowering responses were as represented in Table 6. With increasing intensities of the light,

Table 6. Flowering responses of *Pharbitis* seedlings exposed to 6-hour daylight fluorescent light of various intensities preceding a 10-hour dark period. (Treated on June 21 and dissected on July 4, 1958)

Intensities of daylight fluorescent light	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
10	40	100	4.8	100
25	40	100	4.4	97.5
50	40	100	2.9	77.5
200	38	92.1	1.5	0
500	40	5.1	0.1	0
3000	37	0	0	0

flowering response was reduced. The process taking place in the first 6 hours of the dark period seems to proceed favorably under the light of 10～25 lux, and hardly at all under 500 lux.

Experiment 7. Flowering responses of plants exposed to daylight fluorescent light or far-red light of very low intensities for 16 hours without subsequent dark period were investigated. As shown in Table 7, about one fourth of the experimental plants were induced to flower when exposed to daylight fluorescent light of 5 erg/cm^2./sec. (ca. 1 lux) or far-red light of 20 erg/cm^2./sec. for 16 hours, but no

Table 7. Flowering response of *Pharbitis* seedlings exposed to daylight fluorescent light (FL) or far-red light (FR) of various intensities for 16 hours. (Treated on June 17 and dissected on July 3, 1958)

Light	Intensities of light in erg/cm.^{2}/sec.	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
FL	50	39	0	0	0
	25	39	0	0	0
	5	39	25.6	0.3	0
	0	39	100	5.0	100
FR	300	39	0	0	0
	100	38	0	0	0
	50	37	2.7	0	0
	20	40	27.5	0.3	0
	0	40	100	4.6	97.5

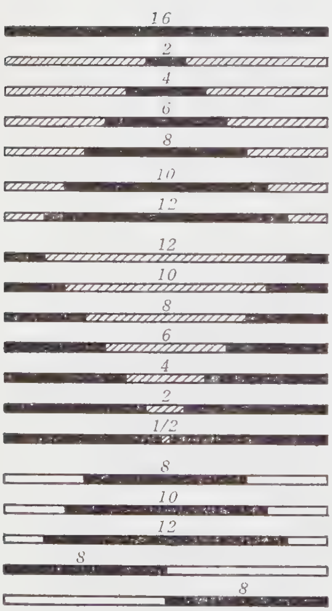
flowering was induced under the former of 25 erg/cm²./sec. and only one out of 37 plants under the latter of 50 erg/cm²./sec.

As had been reported in a previous paper[2)], far-red light of 50～100 erg/cm²./sec. can also strongly inhibit flowering, when given to the plant for 8 hours or more preceding a 16-hour dark period, but the daylight fluorescent light of 50 erg/cm²./sec. can do so only a little. That is, the former strongly inhibits flowering response whether it is followed by the inductive dark period or not, but the latter does not do so when followed by the inductive dark period. This suggests that the flower inhibiting mechanism of the two lights is not the same.

Experiment 8. In a preliminary experiment which is not reported here, low-intensity light of a daylight fluorescent lamp given to the plant following a 12-hour dark period was found to promote flowering responses to some extent. Thus, the last phase of the inductive dark period is, also, considered to proceed under the low-intensity light. In the present experiment, plants were treated according to the schedule shown schematically in Table 8.

Some of the plants which were kept in darkness for only 4 hours initiated flower primordia when the dark period was preceded and followed by 6 hours of illumina-

Table 8. Flowering responses of *Pharbitis* seedlings induced by a 16-hour dark period during which low intensity light (daylight fluorescent light of 10 lux) of various lengths were given at various times. (Treated on June 16 and dissected on June 30, 1958)



Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16	38	100	4.0	68.4
2	40	0	0	0
4	40	30.0	0.3	0
6	36	94.5	2.2	5.6
8	39	89.8	3.0	41.0
10	37	94.6	3.0	35.1
12	39	100	3.9	59.0
12	39	0	0	0
10	38	0	0	0
8	40	0	0	0
6	38	0	0	0
4	40	2.5	0	0
2	38	15.8	0.2	0
1/2	39	84.7	1.8	12.8
8	40	0	0	0
10	40	35.0	0.4	0
12	40	92.5	2.4	27.5
8	39	0	0	0
8	39	0	0	0

darkness
daylight fluorescent light of 10 lux
daylight fluorescent light of 3000 lux

tion by daylight fluorescent light of 10 lux. On the other hand only one out of 40 plants initiated a flower primordium after being exposed to 12 hours of darkness which had been separated into two parts of 6 hours each by the insertion of a 4-hour period of daylight fluorescent light of 10 lux.

This implies that the dark process inducing flower formation is very sensitive to light during the middle phase, but relatively stable during the first and the last phases.

Discussion and Conclusion

In a previous paper[2], we made the assumption that the first process of the inductive dark period can proceed under daylight fluorescent light of 10 lux or under far-red light. This is substantiated by the evidence in the present paper.

The flower-inducing dark reaction consists of several processes. The first process is supposed to proceed under the illumination by daylight fluorescent light of low intensity and the far-red light, but the second or the later process does not. Far-red light given prior to the dark period inhibits flowering through the suppression of the second or the later process and acts to reduce the maximum flowering level, having little effect on flowering responses at lower levels (cf. Experiments 3 and 4). Thus the far-red light preceding the subcritical dark length promotes flower formation and that preceding the maximal inductive dark length inhibits flower formation.

Plants grown under natural daylight are exposed to low intensity light of twilight in the morning and in the evening. During the twilight, the first or the last process of the flower-inducing dark period may proceed. Therefore, the length of twilight of less than a definite intensity must be taken into consideration to define natural night length which is responsible for flower initiation of short day plant, at least in *Pharbitis* seedlings.

Summary

1) If a dark period is preceded by 4 hours of illumination by FL+FR (daylight fluorescent light of 50 erg/cm^2./sec. mixed with far-red light of 120 erg/cm^2./sec.) or by far-red light of 200 erg/cm^2./sec., the critical dark period is shortened by some 4 hours.

2) When plants are exposed to daylight fluorescent light of 50 erg/cm^2./sec. for 8 hours followed by the dark period, the critical length of the dark period is shortened by 6∼8 hours.

3) If the plants are exposed to FL+FR or far-red light for 8 hours preceding the dark periods of various lengths, the flowering is inhibited when the dark period is longer than 14 hours, but promoted when the dark period is shorter than 12 hours but longer than 8 hours. The critical dark length is shortened by some 4∼6 hours.

4) The first process taking place in the first 6 hours of the inductive dark period seems to proceed favorably under daylight fluorescent light of 10∼25 lux,

but hardly at all under that of 500 lux.

5) Critical intensities of daylight fluorescent light and far-red light which can induce flowering when given for 16 hours without giving dark period are 5～25 erg/cm^2./sec. (ca. 1～5 lux) and 50～200 erg/cm^2./sec. respectively.

6) A 4-hour dark period can induce flowering when preceded and followed by 6 hours of illumination by daylight fluorescent light of 10 lux.

It is concluded that the first and probably the last phases of the flower-inducing dark period can proceed under daylight fluorescent light of low intensity or under far-red light.

Grateful acknowledgment is made to Professor S. Imamura for his suggestions and criticisms.

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摘　　要

1) 暗期前近赤外光をほとんど含まない 10 ルックス程度の昼光色螢光燈の光を 8 時間与えると，限界暗期は 6～8 時間短縮される。

2) 上記の光に近赤外光（120 erg/cm^2./sec.）をまぜた場合，および近赤外光のみ（200 erg/cm^2./sec.）を与えた場合は，限界暗期は約 4 時間短縮されるに過ぎない。

3) 暗期反応の最初の 6 時間は 10～25 ルックスの昼光色螢光燈下で充分進行し得るが 500 ルックスではほとんど進行し得ないようである。

4) 昼光色螢光燈，または近赤外の非常に弱い光を 16 時間与えた場合，開花を阻止する最低限の光の強さは，前者で 5～25 erg/cm^2./sec.（1～5 ルックス），後者で 50～200 erg/cm^2./sec. である。

5) 暗期の前後に 6 時間づつ 10 ルックスの昼光色螢光燈の光を与えると，わずか 4 時間の暗期で開花するようになる。

開花に必要な暗期の初期段階は，10～25 ルックス程度の弱光，または近赤外光の下で進行し得るものと考えられる。たゞ近赤外光は，同時に其後の暗期反応を抑制すると考えられるので，暗期前に近赤外光を与えた場合の限界暗期の短縮はそれ程顕著でない。

The Correlation of Nucleus with the Chloroplast-activity*

by Yoshio YOSHIDA**

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For the purpose of obtaining some information concerning the rôle of the nucleus in normal metabolic phase of the cells, some comparative experiments with the leaf cells of *Elodea densa* Casp., which were divided into nucleated and enucleated halves by means of the plasmolytic treatment, have been carried out. In the previous papers[1),2)] it was inferred that the nucleus might have some relations to the catabolic processes of chloroplast, the occurrence of plasmolytic systrophe of chloroplasts, and the mechanical nature of protoplasmic surface membrane. In the present report, however, in order to derive more direct informations concerning the correlation between nucleus and chloroplast, the effects of some metabolic poisons upon such an alteration of chloroplast as previously reported[1)] were investigated.

As is well known, these reagents used here are conspicuously effective inhibitors to various enzymatic systems *in vivo*. Concerning the rôle of the nucleus in the life of a cell, Brachet (1954, '58,[3)] has thoroughly discussed that the nucleus is neither the center of cellular oxidations nor a storehouse of all enzymes, nor their producer, but in some way it might regulate certain steps of many enzymatic activities of the cell, and enucleation is likely to lead to a decrease in various enzymatic reactions, perhaps owing to the lack of some essential cofactors normally produced by the nucleus. Therefore, the comparative investigations upon the effects of these metabolic inhibitors and the influence of presence or absence of nucleus on the chloroplast might be useful in deriving from them some informations for such inference.

Methods

Materials and culture or experimental procedures were fully described in the previous papers. For the metabolic poisons five reagents of mercuric chloride, sodium fluoride, sodium azide, 2·4-dinitrophenol (DNP), and potassium cyanide were used. And they were added respectively to the plasmolytic culture medium (0.2 M calcium chloride aqueous solution), and their experiments were tried on generally with several orders in the range of 10^{-2} to 10^{-5}M. The addition of these metabolic poisons to the culture medium, in general, showed the decrease of the endurable vitality of *Elodea* leaf cells to experimental culture treatment and of the frequency of plasmolytic division of a protoplast. When the concentration of these poisons was too high, the protoplast itself collapsed before the poisonic effect appeared on

* A preliminary note of this study was briefly given in the J. Fac. Sci. Niigata Univ. Ser. II. 2(6) : 221 (1959).

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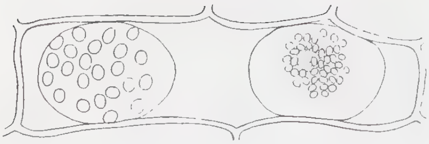

Fig. 1. The protoplast divided in nearly equal halves by means of plasmolytic treatment, cultured for 5 days in plasmolysate.
left: an enucleated half, chloroplasts diffuse and vividly enlarge.
right: a nucleated half, systrophe and senescence of chloroplasts is notable.

the chloroplasts, and if it was too low, it seemed to have no notable influence on the chloroplasts. So the suitable concentrations of each reagent were experientially tried in different ways in the range described above. The *Elodea* young blades removed from the tip of an actively growing stem were soaked into each plasmolysate containing the metabolic poison, then the suitable cells in which a protoplast was divided into two nearly equal halves were sought out, and the effects of such poisons upon the chloroplast alteration were compared with the previously reported[1)] control every day throughout the culture duration.

Results

Control: The results has already been fully described in the previous paper[1)]. In short, the chloroplasts in nucleated halves showed a very remarkable etiolment, shrinkage, and the assimilated starch was decomposed; but on the other hand, in enucleated halves, a significant vivid enlargement and an extreme accumulation of the assimilated starch were noticed, and there was a conspicuous contrast between the chloroplasts in both halves [Figs. 1, and 2, a), and see the previous paper.[1)]].

Mercuric chloride: This reagent had extremely harmful action to the protoplasm, and the protoplast itself collapsed very rapidly. Therefore, it was utterly impossible to continue the culture experiment within a range of the effective concentrations.

Sodium fluoride: The chloroplasts in nucleated halves went through a degenerating processes similar to that in the control, and no more notable changes were recognized. While, in the enucleated halves, recognizable influence occurred on the chloroplasts; the active accumulation of assimilated starch and chloroplast enlargement were clearly hindered. Such effects were most notable at a concentration of 10^{-2}M [Fig. 2, b)], and both starch content and size and form of the chloroplasts were almost kept in the *status quo ante* in untreated normal condition throughout the culture duration. But the etiolment became sluggishly visible after seven to eight culture days.

Sodium azide: This reagent had a considerable influence to protoplasmic characters, and when the experimental materials were treated with the plasmolytic culture medium containing the sodium azide, the plasmolytic grade was very weak, the systrophe[1),4),5)] hardly occurring in any case, and also the occurrence of suitable protoplast dividing was rare. So it was not easy to seek out the objects under the microscope. And also the endurance of the protoplast to experimental culture treatment was weakened in general. It was very difficult, therefore, to follow the effects of sodium azide on the chloroplast in each of protoplasmic halves, and only a few cases were found in the 10^{-4}M experiments. It was the most notable finding in

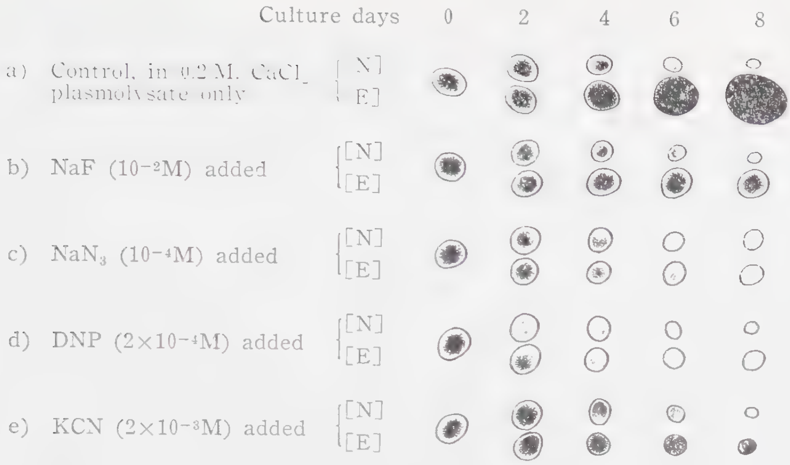

Fig. 2. The effects of respective poisons on the chloroplast alteration and starch formation. [N]: chloroplast in nucleated half. [E]: chloroplast in enucleated half.

these cases, that in chloroplasts of the nucleated halves, the starch dissimilation was a little more accelerated than in the control but the chloroplast shrinkage and etiolment were retarded. And in the enucleated halves, the chloroplast enlargement was clearly inhibited, and moreover, a slight tendency to shrinkage and etiolment were recognized, and also the starch content was decreased as much as in the nucleated halves. In most cases, after all, not so remarkable a contrast as in the control was noted between both halves [Fig. 2, d)].

2·4-dinitrophenol: This reagent also had a deleterious influence on the protoplasmic characters, the systrophe was shifted to a diffusive trend even in the nucleated, the plasmolytic grade and the endurance of divided protoplast was weakened, and also the chloroplast alteration was notably affected. This affection was most active at a concentration of 2×10^{-4}M [Fig. 2, d)]. In the nucleated, the chloroplasts followed senescent process as remarkable as in the control, but the starch disappearance was more rapid and complete than in the control. The chloroplasts in the enucleated halves also showed clearly a considerable shrinking and etiolment as compared with the control, and the starch was almost completely depleted. The inhibiting effect of DNP on the starch formation was extremely striking together in both cases.

Potassium cyanide: The viability of protoplasts was very sensitive to cyanide, and in higher concentration than 2×10^{-3}M, they were completely damaged. Even in lower concentration than that, the deleterious effects of this poison shortened the endurable period of materials, and it was very rare to obtain the suitable materials after five to six days or more. The chloroplasts in nucleated halves showed the entirely identical process of senescence with that in the control, and no more notable alterations were recognized in every concentration of poison. On the other hand, the chloroplasts in the enucleated halves were clearly inhibited the enlargement as remarked in the control. In concentration of 10^{-3}M, the chloroplasts in enucleated were considerably etiolated, but the form and size were kept in the *status*

quo ante. In 2×10^{-2}M, moreover, the shrinking and the etiolment were notable, and they approached to the appearances in the nucleated ones. But the starch content did not decrease, and the chloroplasts gradually filled with starch in proportion to shrinking and etiolment of chloroplast [Fig. 2, e)]. This does not mean, however, any active accumulation of starch but the starch content is rather constant and it is because of the outward condensation by plast shrinking.

Consideration

The grade of plasmolysis is directly controlled by several inner conditions of protoplasm; permeability[6)], viscosity[7)], and adhesion[8)] etc. And recently it was found[9),10),11)] that the sodium azide and DNP brought about a clear decrease of permeability of protoplasm, and also a remarkable weakening action of these metabolic poisons to the systrophe was noticed. In the present experiment also, therefore, the decrease of the plasmolytic-grade, -division, and -systrophe remarked in protoplasm treated with sodium azide and DNP can easily be regarded as the effects of the decrease of water permeability by them.

Mercuric chloride selectively disturbs and eliminates the action of amylase[12)]. According to Ono (1956)[13)], the starch formation was accelerated by the addition of $10^{-3} \sim 10^{-5}$M mercuric chloride to substrate, because the inhibition of amylase, which acted antagonistically to phosphorylase. In the present case, however, the prospects that remarkable starch dissimilation in chloroplast in nucleated parts may be retarded as a consequence of amylase inhibition by mercuric chloride addition and that the nucleus may be correlating to the amylase action could not be confirmed, on account of its intense deleterious action to the protoplast. But other four reagents remarkably affected on such chloroplast alteration as in the control. In nearly every case the chloroplasts in nucleated halves went through a similar degenerating processes to that in the control without any especially greater changes. In a general view, even when the reagent deleteriously affects the cytoplasm, its affection to the chloroplast is not so great. Perhaps, the chloroplasts in nucleated halves seem to be already in the so-called "*residual state*" in which there no longer remains any more affectable points of these inhibitors. Although the chloroplast senescence in the nucleated halves of the materials treated with sodium azide was certainly retarded, yet, perhaps, this seems to be due not to the direct inhibiting effect on the chloroplast senescence by this poison, but rather to the indirect influence that the nucleus, which acts to shift the chloroplast into the senescence, was completely damaged by this poisonic affection and in consequence the protoplast approached to almost similar state to that of enucleated halves.

According to Porter (1953)[14)], the sodium fluoride selectively inhibits the action of phosphatase. Ono and Konagamitsu (1957)[15)] also reported that the existence of acid phosphatase inhibits directly and indirectly the formation of starch in leaves, and they pointed out, therefore, that the starch formation was rather accelerated by the addition of the sodium fluoride ($10^{-2} \sim 10^{-3}$M) to substrate. But in the present case, on the contrary, the abnormal accumulation of starch in the chloroplasts in the enucleated halves was completely hindered and the chloroplasts were left in the

same state as in normal ones without any more alteration. Perhaps the sodium fluoride attacked some points in the metabolic system of chloroplast in the enucleated halves with similar effect to the regulation by the existence of nucleus. In fact Bishop and Gaffron (1958)[16] recently reported that the effect of sodium fluoride on photosynthesis of green algae was found to be very complex; in some instances complete inhibition occurred, whereas in other experiments there was no inhibition.

It was found out by Clifton (1937)[17] that the DNP has a marked inhibitory effect on starch formation. But recently Berke and Rothstein (1957)[18] noticed that the DNP acts by increasing the rate of dissimilation rather than by inhibiting the assimilation. Really, the fact that not only the starch was never newly stored in the chloroplasts in materials treated with DNP but even the pre-existing starch was depleted very rapidly and almost completely, can be well understood in such connection. According to Brachet (1951)[19], the DNP leads to a blocking of the normal coupling between oxidation and phosphorylation, and interrupts various synthetic processes. And it was discussed that as the non-nucleated amoeba would behave just like cells treated with DNP, the nucleus plays some parts in the synthesis of the coenzymes necessary for this coupling. In the present case, however, its affections was utterly opposite; it was shown that the existence of nucleus brought about a degenerating influence similar to the DNP-treatment on the chloroplasts.

In the previous paper[1] it was inferred that the nucleus might play an important rôle in the catabolic processes of chloroplast. Indeed recently Ueda (1959)[20] confirmed that the separated chloroplasts lost in a few days the accumulated starch in them after restitution into the cytoplasmic mass containing nucleus, and in consequence, it was concluded that the nuclei are contributing to a successive degradation of assimilated starch, so as to make it available for further metabolic transformations. Brachet (1958)[3] thoroughly discussed that in non-nucleated Amoeba cytoplasm the carbohydrate metabolism is deficient[21], and its more likely explanation might be found in a suppression of the formation of very important coenzyme, DPN, which is synthesized in the nucleus,[22),23),24] and that the activity of some of the cytoplasmic enzymes might be regulated by the production and release of coenzymes from the nucleus. And Arnon *et al.* (1958)[25] also reported that the photosynthetic phosphorylation by isolated chloroplasts is coupled with TPN reduction. After all, the catabolic processes of chloroplast are probably dependent on the supply of some necessary co-factors by nuclear action, though the chloroplast has an autonomy[26),27] in a considerable degree. And the normal metabolic equilibrium of chloroplast as a whole will be controlled by such an antagonistic relation. The abnormal enlargment and starch accumulation of the chloroplast in enucleated halves seem to be the consequence of the disturbance of the metabolic balance owing to the retardation or absence of the decomposition by the deficiency of some co-factors normally supplied by the nuclear influence.

These poisons used here are powerful inhibitors of the various enzymes[28]. But they are not always absolutely specific and affection is very extensive and complicated. It is very difficult, therefore, to conjecture, from these results alone, the precise points attacked by these inhibitors in various metabolic processes of the

chloraplast. The comparison, however, between the control, and the results of inhibitor treatments clearly shows the interesting fact that the influence of the presence of nucleus on the chloroplasts resembles the affection of these inhibitors on the chloroplasts freed from the nuclear control. The present result suggests that the nucleus is never normally functioning under such an abnormal and compulsive condition as a plasmolytic treatment. Probably the nucleus is apt to become necrobiotic in its function in such a condition, and such necrobiotic nucleus will shift the metabolic equilibrium of the chloroplast toward the necrobiotic senescence in consequence of a disturbance of normal metabolic system similar to the affection of some poisons. And even if the chloroplast is brought under such a condition no remarkable necrobiosis occurs so easily in the intact chloroplast so far as it stands outside of the influence of nucleus. In fact, the vital activity of chloroplasts in nucleated or enucleated halves was confirmed already by means of the silver-nitrate reduction[29]. According to Maruo *et al.* (1953)[30] and Ono (1956)[13] the Ca^{++} ion inhibits the starch formation. And also it has been known that the plasmolytic condition strongly depresses the photosynthesis [31),32),33] and, in a green alga, the respiration[34], and it was explained as owing to the dehydration from plasma. But as already mentioned above and in the previous paper[1], the result of this study suggests that the influence of plasmolysis on the chloroplast function is not direct but indirect, through necrobiotic affection of nucleus harmed by plasmolytic treatment, because even in the same experimental culture with $CaCl_2$ plasmolysate the chloroplast is able to keep an active ability of the starch formation and others so far as it stands outside of the influence of nucleus. And also, as is well experienced, in inactive plants grown in shadow, under-nurishment, and in other unfit conditions, the decomposition and disappearance of assimilated starch in chloroplasts is frequently very sluggish even in a dark condition. And it will be conjectured that such phenomena also may be related to the decrease of nuclear activity which plays an important rôle in the catabolic processes of chloroplast.

Various questions are, however, yet far from settled and new experimental approaches are obviously necessary for us to make more definite progress in the study of the correlation between the chloroplast and the nuclear function.

Summary

In order to gain some informations concerning the function of the nucleus and its correlation with other cell elements in normal metabolic phase of the cell, the effects of some metabolic poisons on the chloroplasts in the protoplasmic halves of an *Elodea* leaf cell, which were obtained by means of the plasmolytic treatment, were compared with the influence of presence or absence of nucleus in them. And from extensive considerations, the following conclusions were obtained;

1) The nucleus is probably apt to suffer from a delicate necrobiosis under the abnormal and compulsive condition such as a plasmolytic treatment, and such necrobiotic nucleus affects the normal metabolic equlibrium of chloroplast, and shifts it toward the necrobiotic senescence similar to the effects of some poisons.

2) The chloroplast has a considerable autonomy, but its catabolic processes are

probably dependent on the supply of some necessary co-factors by nuclear action.

3) The depression of photosynthetic activity in the plasmolytic condition is not owing to direct affection to the chloroplast function but indirect influence through the necrobiotic nucleus.

I wish to express my gratitude to Assist. Prof. R. Ueda in the Tokyo University of Education, Prof. T. Soma in the Faculty of Education of this University, and Prof. H. Takada in the Osaka City University for their valuable suggestions and criticism. And I am also grateful to Prof. N. Kamiya in the Osaka University for his hearty encouragement, and to Prof. A. Yuasa in the Tokyo University for his helpful advice in preparing this manuscript.

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摘　　　要

細胞の正常代謝期における核の機能およびその他の細胞要素との相関性の問題について何らかの知見を得んがために、[illegible]代謝毒の影響を核の存否の影響と比較検討した。そして色々な考察の結果から結局次のような事柄が推論せられた。すなわち：

1. 核というものはおそらく原形質分離というような異常な強制的条件下では微妙な病変に罹りやすいものであって、そのような[illegible]にそれを傾けるものである。

2. 葉緑体はかなりの自律性を有するもので，そのような異常条件下でも核の影響外にある限り強くその機能を[illegible]し得るものである。しかしその[illegible]らく核の作用による何か必須の補助要因の供給に依存しているものである。

3. 原形質分離的条件による光合成能の低下は従来いわれたような葉緑体機能への直接的影響のせいではなくて，病変核を介しての間接的な[illegible]

Variation of Photosynthetic Activity with Aging of Leaves and Total Photosynthesis in a Plant Community**

by Toshiro SAEKI*

佐伯敏郎*： 葉令による光合成能力の推移と植物群落における全光合成

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Varying photosynthetic activity of leaves accompanied by aging has successfully been studied with coniferous and broad-leaved trees[1),2),3),4)]. With herbaceous plants, however, only a few, rather fragmental data[5),6)] are so far available, probably because of the promptness of the variation of photosynthetic activity. The author will present here some results measured with herbaceous crop plants. On the other hand, the CO_2-fixation of plant population as a whole implies summation of the photosynthesis of all the constituent leaves, different in activity, such as sun and shade leaves in trees, or younger and older leaves in herbaceous plants. Moreover, the way in which such functionally different leaves contribute to the total photosynthesis in a plant community will be discussed.

Variation of photosynthetic activity with aging of leaves in some herbaceous plants

Green gram (*Phaseolus viridissimus*) and buckwheat (*Fagopyrum esculentum*) were used as experimental plants. Green grams were sown at an interval of 10 cm. in square disposition. The sprouted young plants were thinned properly so that no mutual shading of leaves might occur. Magnitude of photosynthetic rate measured at saturated light intensity (25 kilolux), constant temperature (25°±1°) and 0.03 volume per cent. of CO_2 was defined as photosynthetic capacity. Measurements of photosynthesis were made using detached leaves by the same method as reported in a previous paper[2)]. After ascertaining stomatal aperture of the leaves microscopically, the leaves were instantly subjected to duplicate photosynthetic measurements. After these the stomata were reexamined. The mean value of these apparent photosynthetic capacities was plotted against days after sowing in Fig. 1, except for values obtained in the leaves whose stomata had not been well opening. In older stages of growth, keeping the stomata open during each measurement was so difficult, that only a limited number of values were available. As seen in Fig. 1, when taking leaves at a definite number of node, the varying pattern of the photosynthetic ac-

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tivity with aging is not essentially different from that of tree species[2),3)]. The most remarkable difference between the two types of plants lies in the length of life of individual leaves; activity in ordinary broad-leaved trees, in general, remains over full growing season, and the maximal efficiency, for example, of *Zelkowa serrata*, a deciduous tree, persisted for two and half months,[2)] while in this green gram and rice plants[5)] the life of leaves was markedly shortened, and maintenance of the highest activity did not exceed even one week. In stead, a higher level of activity in this plant was notable compared with the activity so far measured in trees[2),3),7)].

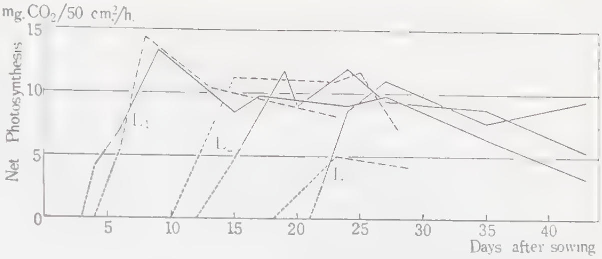

Fig. 1. Variations of photosynthetic capacity in *Phaseolus viridissimus* leaves accompanying with leaf age. Solid lines: solitarily grown plants (sown at Aug. 8, 1956). Broken lines: mixed planting with *Fagopyrum esculentum* (sown at July 4, 1957). Dotted line indicates the day of leaf appearance.

Similar pattern was also confirmed in the buckwheat plants which were planted alternatively with green gram plants in square disposition, intervals between plants being held 10 cm. (see also Iwaki[8)] 1959). Buckwheat leaves did not receive any shading effect of green gram leaves, because main stems of the former elongated more rapidly than those of the latter, though a slight self-shading still occurred. Variations of photosynthetic capacity with aging in the measured leaves of the buckwheat plants are summarized in Fig. 2. Compared with green gram, it will be seen, buckwheat is characterized by its further shorter plastochrone (2–3 days) and life of leaves. For this promptness of senescence, the shading of the lower leaves may be responsible, as indicated in soybean by Kusumoto[6)] with two comparable plants grown solitarily and in community state.

In our mixed planting, green gram plants gradually fell under the shade of faster elongating buckwheat plants, and the third leaves of the former unfolded and expanded under a relative light intensity from about 50 to 85 per cent. Such circumstances were reflected in the level of the photosynthetic capacity, namely, it was significantly different from that of normal leaf at sunny place and comparable with, so to speak, shade leaf type. The maximal activity has not attained to more than 5 mg. CO_2/50 sq. cm./h. (Fig. 1), and the leaf thickness expressed as leaf dry weight per unit leaf area was less than 80 per cent. of the thickness of the normal leaf devel-

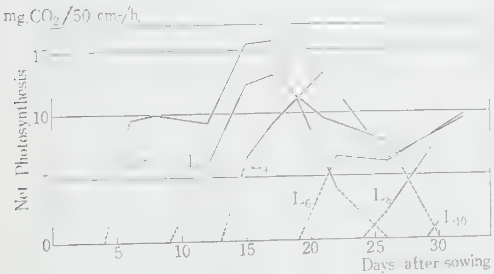

Fig. 2. Variation of photosynthetic capacity of *Fagopyrum esculentum* leaves accompanying with leaf age. Leaves were numbered upward along main stem. Oddly numbered leaves were not measured. Dotted lines indicate the times of leaf appearance and falling.

oped in full sunlight.

Variety in photosynthetic activity among leaves of individual plant

From foregoing, the leaves are, as photosynthetic agency of a plant community, expected to have a specific distributional pattern, determining not only the vertical light distribution, but also the gradient of functional activities such as photosynthetic rate and respiratory one. This was more clearly shown in an experiment with *Celosia cristata*. Seeds of this plant were so densely sown (June 20, 1955) in an experimental plot that a keen intraspecific competition had took place, and consequently, when measurements were made, only a small number of the original seedlings were left alive. Photosynthesis measurements were made in a material plant bearing 48 leaves and an inflorescence, about 6 cm. in length, from Sept. 7 to 12, 1955. The area of the several leaves near the inflorescence was so small that the leaves could not be used for the measurement. When leaf lamina was too broad to use, only its separated small part with petiole was used. Light-photosynthesis curves measured with some sample leaves were shown in Fig. 3. As expected from the foregoing experiments and results of other workers[3),5),6)], activities of the lower leaves were rather low. The lowest leaf was the lowest in activity, and the inclination of the curve at weaker light is also smaller than that of other leaves, corresponding to its chlorophyll deficient, yellowish colour. The young leaves (the 37th to 39th leaf numbered from the lowest) were at the highest level of activity. This may probably imply that they were already matured though their sizes were

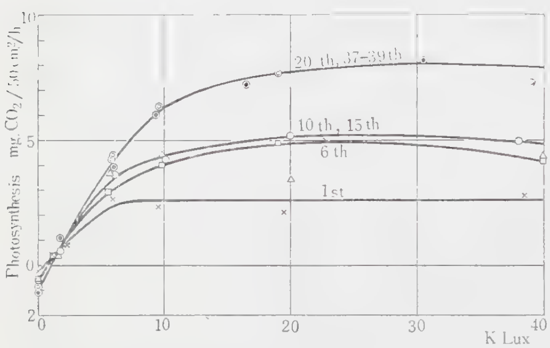

Fig. 3. Light-photosynthesis curves in *Celosia cristata* leaves at vertically different positions of stem. The leaves were numbered upward from the lowest one left alive.

Table 1. Leaf size, photosynthetic capacity and respiration in the leaves at different stem positions of *Celosia cristata* plant in a community state. The leaves are numbered upward from the lowest leaf left alive.

Leaf number	Leaf size (unilateral) sq. cm.	Photosynthetic capacity mg. CO_2/50 sq. cm./h.	Respiration at 25° mg. CO_2/50 sq. cm./h.
1st (yellowish)	45.6	2.6	0.23
6th (green)	42.0	4.9	0.58
10th (green)	52.1	5.2	0.57
15th (green)	61.3	—	0.61
20th (green)	63.1	8.3	0.87
37th-39th (green)	8.5	8.3	1.09

very small (Table 1.). Besides, comparison of these curves will provide a significant correlation between photosynthetic activity and respiratory one. It is summarized in the Table with the data on leaf size.

Relation between arrangement of functionally different leaves and total photosynthesis of a plant community

Productivity of a plant community is sustained by photosynthesis of the constituent leaves, different in activity as indicated above. It must, however, be remembered that the active leaves are, in general, situated at the upper bright parts and the inactive ones at the lower parts of main stem. It is also the case in the trees which carry sun and shade leaves. The significance of such characteristic activity gradient on the productivity of the whole plant community should be clarified by considering it in connexion with corresponding vertical illumination gradient. It has already been emphasized that in lignosa and herbosa only a small fraction of light can reach the vegetation floor[9]. The lower leaves, owing to limitation imposed by such low illumination, can expect higher productivity only when lowering the respiratory loss, because gross photosynthesis of these leaves in weak illumination lies in the same highest level as in upper leaves[10]. Lower respiratory activity is generally associated with lower photosynthetic activity (see Table). So far as plants construct a dense plant community, such high activity as displayed only at high light intensity should, for the deeply shaded lower leaves, be scarcely any use. Furthermore, it may also be stressed that thinness of shade leaves, or of old leaves being deprived of nutrients for upper young growing parts is favourably efficient for high productivity of the plant community as a whole. Consequently, it is concluded that the characteristic arrangement of functionally different leaves both in herbosa or lignosa is most effective for yielding higher productivity.

The inclination angle of light-photosynthesis curve in weak illumination is determined largely by chlorophyll content of leaves, which attains in general to a level high enough to provide a maximal efficiency in such an illumination[10]. Therefore, different leaves of a plant display in weak illumination a similar photosynthetic activity, except for very young and old yellow leaves, as realized in *Celosia* (Fig. 3) and soybean[6]. So, the most of the leaves practically work under the light condition in a dense plant community along one and the same photosynthetic curve which is obtained in the upper leaves with high activity. This can be supported with the experimental data presented by Yamada *et al.*[11] Though this may not be adopted to very young or old leaves, their participation in total photosynthesis of the plant community is negligibly small. As a conclusion, for the purpose of estimating the total photosynthesis of a dense plant community, it is very reasonable and also convenient to adopt the light-gross photosynthesis curve in an active leaf such as just matured, and the mean respiration rate of all constituent leaves.

Summary

1. Variation of photosynthetic activity with aging of leaf was pursued, using green gram (*Phaseolus viridissimus*) and buckwheat (*Fagopyrum esculentum*) leaves at

early stages of growth. Compared with trees so far measured, these herbaceous species are characterized by very brief life and duration of the highest level in photosynthetic activity of each leaf, though the form of varying pattern was the same as in trees.

2. In a plant community, as indicated with *Celosia cristata*, the constituent leaves different in activity are arranged on a specific pattern, which is most favourable to the total photosynthesis of the plant community, if considered in connexion with light distribution within the plant community.

3. For estimating total photosynthesis of a dense plant community, it is reasonable and also convenient to adopt the light-gross photosynthesis curve obtained in a upper leaf with high activity, and mean respiration rate of all constituent leaves.

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摘 要

生育初期のヤエナリ (*Phaseolus viridissimus*) とソバを材料として，葉令による光合成の能力変化を調べた。前報に示したケヤキその他木本の測定結果と比較した場合，これら草本における光合成能力の時間的推移の形はほぼ同じであるが，葉の寿命が短かく，光合成能力の最高値もごく短時間しか持続しない。ケイトウの葉で示されたように，草本群落では光合成能力の高い若い葉が上部に，老令でかつ陰葉化した葉が下部につく。森林の場合には葉令は同じでも樹冠は陽葉と陰葉に分化するから結果的に同様な光合成能力の垂直こう配が成り立つ。このことは群落内の光の垂直分布と結びついて群落全体としての生産に有効に働らく。また密な植物群落全体の光合成を見積るためには上部の活性のよい葉でえられる真の光合成曲線と全葉の平均呼吸を代表にえらぶだけでよい。

A Note on the Internal Callus Formation in Radish Roots

by Kotaro WATANABE*

Received June 11, 1959

With respect to the thickening growth of roots, those of radish (*Raphanus sativus* var. *hortensis*) have been anatomically observed by Golinska[1], Namikawa and Sawamura[2], Hayward[3], etc. The succulent portion of this plant comprises two portions, root and hypocotyl. In both of these parts their development is taken place in a fairly similar way: the thickening growth is carried out by the activity of cambium ring and of many secondary cambia, which arise in the secondary xylem. The activity of general division and growth of the parenchyma in the secondary xylem are also incorporated.

In an anatomical comparison between a horticultural variety (Moriguchi-Daikon) with a long and slender root and another variety (Sakurajima-Daikon) with a gigantically thickened root, the present author has found that in the latter variety the secondary cambia develop vigorously and the xylar elements are scarcely lignified except vessels and those in their vicinity. These observations agree with those made by Soeding[4] in the roots of *Brassica Napus*. He compared anatomically a variety (var. *napobrassica*) which has a thick root with another variety (var. *raps*) with no such a thickened root. Moreover another resemblance has been found between *Raphanus* and *Brassica*. In the early stage of the development, a small hole arises in the center of the root and sooner or later this hole is found being filled up with parenchymatous cells from the surrounding tissue. A detailed observation on this phenomenon will be stated here.

Three horticultural varieties of *Raphanus*, Moriguchi-, Sakurajima-, and Minowase-Daikon, grown under usual cultural conditions were observed. Of these the Minowase-Daikon is one of the most common radishes in Japan. In each case the succulent portion was examined in definite intervals from the beginning of germination to the harvest time. Sections, fresh or fixed, were stained with Delafield's haematoxylin, and mounted in glycerol or balsam.

The radish root has a diarch actinostele. In the xylary development, the metaxylem continue to differentiate between the two protoxylems so that the vessels of the primary xylem come to arrange in one row. Then the vessels of the secondary xylem develop on both sides of the primary xylem strand in the neighbourhood where the two metaxylems have been fused.

In the Moriguchi-Daikon, a cavity formed lysigenously was first observed in a

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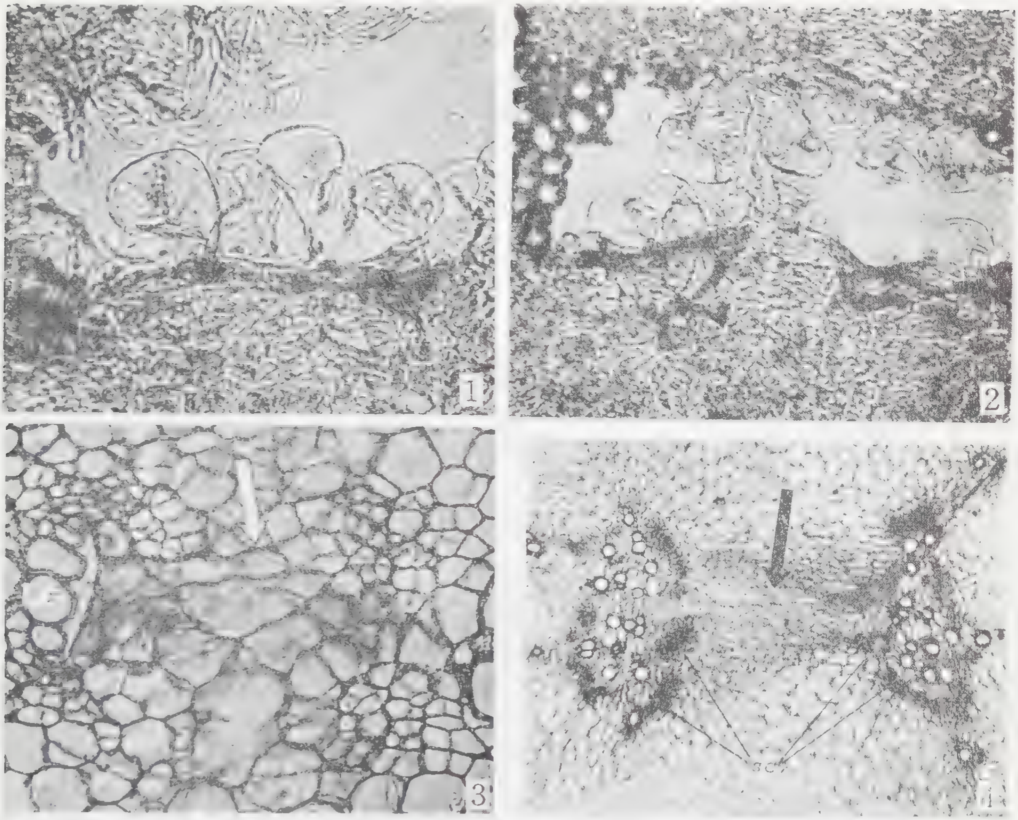
root of the seedling of 19 days old after the sowing. At that time the maximum diameter of the root was about 3 millimeters. Such a cavity is recognizable much clearly in a 24 day old root (max. dia. 4 mm.). Usually the cavity appears in a place between the primary xylem and the secondary one. Frequently it arises also in the secondary xylem closely adjacent to the primary xylem. Soon the cavity grows into a small hole through breaking of the surrounding tissue. The brownish remnants of the ruptured cells are seen attaching to the wall of the hole. The vessel strand of the primary xylem is often broken, being separated in two or more parts by the development of the hole.

In the Minowase-Daikon, a cavity was first seen in a root of 24 days old after the sowing (max. dia. 4 mm.), and in the Sakurajima-Daikon it was recognized about 40 days after the sowing (max. dia. 10 mm.). However, in the Moriguchi-Daikon, as mentioned above, the cavity was found in a 19 day old root. In the last variety the thickening growth extends most rapidly towards the root tip. The beginning of the above-mentioned lysigenous cavity, therefore, seems to have an intimate relation to the rapid thickening growth in the early stage of development. It is difficult to decide exactly at what part of a root the split or cavity first appears, but usually the split seems to occur at the middle or rather upper part of the thickened portion along the root axis.

After the formation of the small cavity or hole, callus cells develop toward the cavity from the cells of the surrounding layer of this cavity (internal callus formation). In Figs. 1 and 2, the internal callus formation observable at the basal part of the hypocotyl is shown, where the structure is almost identical with that of the root. Round, rather large thin-walled cells appear at the parts of the cavity wall (Fig. 1), where the coloured disintegrated cells are absent. The phenomenon reminds us somewhat of "tylosis" or "tylosoids". The cells grow and devide in making a parenchymatous tissue (Fig. 2) and the cavity is filled up with this tissue (Fig. 3). In most sections of the root, it is usual to observe either a cavity formed lysigenously or that closely filled with parenchymatous cells, and a transitional state between these is rarely seen. Accordingly it is conceivable that the hole can be filled up with parenchymatous cells very rapidly after the formation of callus cells. The parenchymatous tissue in question originated from the callus cells continues further cell division, and attains, at the harvest time, the size of several hundred micra to about 2.5 millimeters in length in transverse section (Fig. 4). During earlier stage of the thickening growth, it is highly probable to think that the break down of tissue (cavity formation) and the callus formation are repeatedly taken place by turns.

In mature radish, the tissue derived from the callus is macroscopically observable at the middle and the lower parts of the succulent portion. At the upper part, however, it is usually difficult to recognize the presence of the tissue as such unless the microscope is used, since lignified secondary cambia and small parenchymatous cells enclose the tissue.

In a sample of the Moriguchi-Daikon, 78 days old, a cavity like a pin-hole was found in the lower part, near the end of the succulent portion. A few callus cells



Process of callus formation in the central portion of radish roots.

Figs. 1-2. Sakurajima-Daikon: the basal part of the hypocotyl, about 40 days after the sowing. Figs. 3-4. Moriguchi-Daikon: 33 and 105 days (harvest time) after the sowing. All show transverse sections.

Fig. 1. Appearance of callus cells from the cavity wall. ca. ×220

Fig. 2. Callus cells filling up the cavity. ca. ×145.

Fig. 3. Showing a callus tissue (↑). px—primary xylem. ca. ×150.

Fig. 4. Callus tissue (↑) which developed well. sc—secondary cambia. ca. ×16.

are seen in this cavity. No growth and division seem to occur in them. Moreover, the walls of cells surrounding the cavity are undergone lignification, thus a new callus formation being restricted.

In spite of his precise description on the root of *Brassica*, Soeding[4] reported the filling up of the hole as being due to the rapid intrusion of the surrounding parenchyma, without noticed the callus formation. As Soeding stated, the occurrence of the small hole can presumably be attributed to the stronger growth in the neighbourhood of the cambium ring than in the central portion. In the Shogoin-Daikon, another horticultural variety of radish, Fujita[5] reported that the pith tissue that develops only at the upper part of the hypocotyl can be destroyed mechanically by vigorous growth of the surrounding portion in making a cavity there. This cavity remains as a large hole, not being filled up with callus cells. Here, the conclusion may be made that the process of the hole formation is the same in nature in all

cases of Sakurajima-Daikon, Minowase-Daikon, Moriguchi-Daikon and Shogoin-Daikon. In usual cases, the hole is repaired sooner or later by the formation of callus tissue, but at the upper part of the hypocotyl, where the pith tissue is present, the callus formation does not keep its pace with the growth of the tissue surrounding the hole, and may remain as a large one. Such a large hole can often be seen, especially, in varieties which develop a large thickened hypocotyl, such as Sakurajima-Daikon and Shogoin-Daikon.

In closing the brief note it may not be superfluous to add that the process of wound healing in grafting is a sort of inner callus formation.

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摘　　要

ダイコンの根の肥大初期には，おそらく周辺部の生長に中心部の生長が追いつかないため，中心にある一次木部の近辺に破生間げきに由来する小さな孔が生じる。この孔はまもなく，その周壁から生じた多くのカルス細胞の生長，分裂，ゆ合によってできた柔組織でうずめられる。カルス組織の介在，発達の結果，一次木部に接してその両側に発達した二つの二次木部道管群は，直径的にたがいにはなれて位置するようになる。

Ecological and Physiological Studies on the Vegetation of Mt. Shimagare

III. Intraspecific Competition and Structural Development of the *Abies* Forest**

by Sumio KUROIWA*

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Received May 29, 1959

Intraspecific competition of plants has been investigated not only by silviculturists and agriculturists with practical concern for sound management of the forest and crop field, but also by ecologists with purely scientific interest since the classical studies of Clements *et al.*[1] Recently Kira *et al.*[2,3,4] have succeeded in mathematical formulation to elucidate some of the problems concerned. On the other hand, studies on the intraspecific competition have been advanced on the basis of dry matter production of plants by Boysen Jensen[5,6], Satoo[7,8,9], Iwaki[10], etc.

As for forests, however, it has not yet been pursued how the forest stand changes its structure successively with its development through the intraspecific competition, mainly because of difficulty to find out a serially aged stand in an identical macro-environment. For the study of this problem the *Abies* forest in Mt. Shimagare seems to be a very fitted object, as it consists of several Forest Units of serially aged trees which are arranged spatially in a characteristic order as reported in a previous paper[11].

In the present paper the author will discuss the frequency curves of tree measures of the *Abies*, such as height, trunk diameter and tree weight, and the tree density, then will demonstrate the development of productive structure of tree classes in the stand.

Method

As reported in a previous paper[11], *Abies Veitchii* hardly differed from mixed *A. Mariesii* in tree size and density throughout the stand development in this region. Therefore, without distinction of these two species, trunk height and diameter breast high (or basal diameter at young stand) were measured for every tree which existed in the sampling area of Forest Unit IV and V, besides the determination of tree density. In young stands, several standard trees were selected from represen-

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tative tree classes, dominant, intermediate and suppressed. These trees were divided, after the stratifying clip method (Monsi and Saeki[12]), into needles, branches, trunk and roots in several strata, then each of them was weighed respectively in order to illustrate the productive structure of individual tree and furthermore of the young stand as a whole.

Frequency distribution of tree measures

In regard to the frequency distribution of trunk height, the young stand showed somewhat asymmetric curve having the mode at the smaller class, while the mature

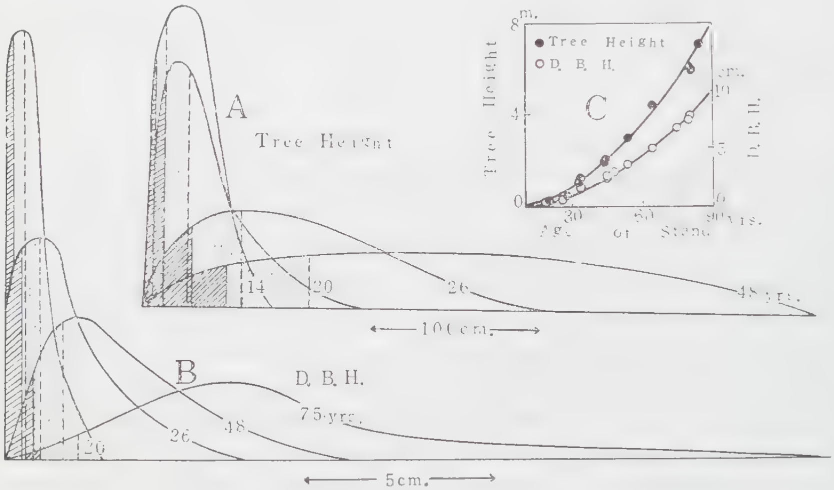

Fig. 1. Development of frequency curves of tree height (A) and diameter breast high (B) with stand age. The absolute tree sizes of the smallest survivors at each stand age (the starting points of the frequency curves in A and B) are shown in Fig. 1-C. Forest Unit V in the *Abies* forest of Mt. Shimagare.

stand showed a roughly normal curve (Fig. 1-A). As for trunk diameter, the asymmetric frequency distribution was also seen in the young stand and this character was maintained throughout the stand development (Fig. 1-B). Such an asymmetric curve and normal one were designated by Kira *et al.*[2] as L-shaped and N-shaped type.

In constituent trees of the young stand, the relationships of basal diameter (D) to needle dry weight (F) and to tree dry weight (W) can be formulated as follows (ref. Fig. 2).

$$F = F_0 D^f \ldots\ldots\ldots\ldots\ldots (1), \text{ and } W = W_0 D^w \ldots\ldots\ldots\ldots\ldots (2)$$

where F_0, W_0, f and w are constants (f and w were 3.0 and 2.7 in the observed case). Assuming these relations to be applied also to mature stand, the frequency distributions of F and W in each developmental stage are able to be derived from distribution of D in the corresponding stage. The F-frequency curve which is composed

with plotting $F_0D^{3.0}$ on cubic scale (see Formula 1) becomes the same L-shaped curve as the D-frequency curve in Fig. 1-B, and consequently the F-frequency curve should become on an arithmetic scale an extremely L-shaped one. Furthermore, on arithmetic scale, the F-frequency curve has to skew in higher degree than the W-frequency curve, because the value of f in Formula (1) was larger than the value of w in Formula (2). These extremely L-shaped frequency distributions of needles weight and tree weight are kept throughout the stand development, because the diameter distribution was continuously of L-shaped curve.

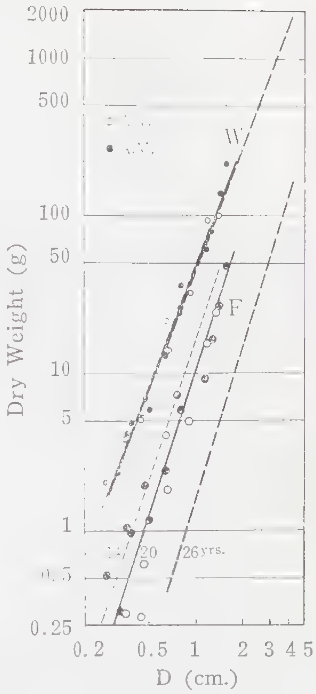

Fig. 2. Linear relations of basal diameter (D) to tree dry weight (W), and to needles dry weight (F) on a log-log coordinates. Young *Abies* stands of Forest Unit V. A.V. $=$ *A. Veitchii*, A.M. $=$ *A. Mariesii*.

As to the intraspecific competition in weight growth Kira *et al.*[2),3),4)] have found the following: (1) the N-type of weight frequency curve in seeds and yearlings gradually skews into the L-type with the stand development, and (2) the time length required to reach the L-type is shortened with increasing plant density. Regarding the serially aged *Abies* stand started from an overpopulated young growth[11)], it seems to be natural that the severe intraspecific competition concomitant with overpopulation occurs so early that skewing of frequency curve of tree weight has already been finished in the youngest growth which was unfortunately not surveyed, and consequently that the above mentioned frequency curves of the estimated tree weight showed only the extreme L-type. The N- or somewhat L-shaped frequency curve throughout the *Abies* stand development, however, seems to be owing to the cooperative interaction in stem elongation among the constituent plants, in agreement with the results analyzed by Kira *et al.*[2),3),4)] and with the data presented by Yamada[13)].

With regard to the characteristic measures of tree, the weights of needles, branches and trunk, and the basal area, it may be silviculturally an interesting problem whether or not the total stand value based on the frequency histogram is identical to the product of the tree density and the value obtained in an average tree for the D-frequency distribution. Satoo and Senda[9)] have found a difference between these two values regarding needles and branches of *Chamaecyparis obtusa*, though they did not mention a statistical significance of the difference. The significance will be ascertained with clarifying how the diameter size relates to the other characteristic measures of tree. For instance, in case of needles weight, tree weight and basal area in young *Abies* stand, it will be ascertained by using the relations of diameter to needles weight (Formula 1), to tree weight (Formula 2) and to individual basal area ($\pi D^2/4$). According to the theorem in inequality, the fol-

lowing relations should be established,

$$\left(\frac{P_1D_1^3+P_2D_2^3+\cdots+P_nD_n^3}{P_1+P_2+\cdots\cdots+P_n}\right)^{\frac{1}{3}}>\left(\frac{P_1D_1^{2.7}+P_2D_2^{2.7}+\cdots+P_nD_n^{2.7}}{P_1+P_2+\cdots\cdots+P_n}\right)^{\frac{1}{2.7}}>$$

$$\left(\frac{P_1D_1^2+P_2D_2^2+\cdots+P_nD_n^2}{P_1+P_2+\cdots\cdots+P_n}\right)^{\frac{1}{2}}\quad\cdots\cdots\cdots(3)$$

where P_1, P_2,$\cdots\cdots P_n$ and D_1, D_2,$\cdots\cdots D_n$ are tree number and mean diameter size in each diameter class, respectively. The first term of Inequality (3) indicates an average tree diameter for the frequency curve of estimated needles weight, the second does an average one for the frequency curve of estimated tree weight, and the third an average for frequency curve of individual basal area. Therefore, a tree diameter is the largest in the average for needles weight frequency curve, the middle in the average for tree weight frequency curve, and the smallest in the average for basal area frequency curve. The larger the diameter, in general, the larger both the needles weight and the tree weight. Accordingly, it can be concluded that the tree weight and needles weight of an average tree for needles weight classes are the largest, those of an average tree for tree weight classes are the middle, and those for average one for basal area classes are the smallest. Both the needles weight and tree weight per stand will be underestimated, if the assesment is made based on an average tree for the D^2-frequency distribution. The measures of the average tree for basal area can directly be applied as the true average for the other tree measures, only when these measures are expressed as linear function of D^2. In the same manner, it may also be concluded for Satoo and Senda's data[9] that the calculation based on an average tree for basal area causes a slight underestimation of the needles weight and branches weight per stand, because these values of *Chamaecyparis* trees were linearly related to $D^{2.6}$ and $D^{2.2}$, respectively.

Tree density

Self-thinning of *Abies* trees under severe intraspecific competition was discussed in a previous paper[11], and there existed a linear relation between the logarithmic values of tree density (ρ) add Plot Nos. within each Forest Unit, except for the overmatured stand. As Plot Nos. can be replaced directly by stand age (t),

$$\rho=\rho_0\exp(-r_0t)\quad\cdots\cdots\cdots(4)$$

where ρ_0 is initial density and r_0 is a constant. Then, $\rho'=-r_0\rho$. This means that the annual mortality of tree was constant independently of stand age (the mortality in Forest Unit IV and V was ca. 7.5%). On the other hand, the mortality in the smallest tree class could be estimated to be equal to the ratio of the marked area to the whole area of each frequency curve of Fig. 1-A and B, provided that the smaller the tree size the easier the tree is defeated in competition. The length of the abscissa of each area concerned was determined to be equal to the tree size increment of the smallest survivor per stand for 5 years (hatched) or 10 years (hatched+dotted).—This tree size increment was computed by using the relation between the stand age and the tree size of the smallest survivor (Fig. 1-C). The annual mean

mortality thus calculated was about 3% on 5 years basis and about 4% in 10 years basis in all observed stands except for 75-year-old stand. The calculated mortality in the smallest tree class is, therefore, approximately only a half of the mortality gained from the annual decrease of tree density in the field. This indicates that the mortality in the smallest class is nearly equal to that in the other larger classes, and that the mortality at which the intermediates will be defeated by the dominants will decrease with size increment of constituent trees.

In the standard trees (average trees for basal area) reported in Tab. 3 of a previous paper[11], the following linear relations are found out between tree age (t), diameter breast high (D) and tree height (H).

$$D=dt-d_0 \ldots\ldots\ldots\ldots\ldots\ldots (5), \text{ and } H=ht-h_0 \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

where d, d_0, h and h_0 are constants, with inevitable exception of trees younger than ca. 14 years whose growth is depressed in the shadow of matured stand canopy of the upper Forest Unit[11]. When these equations are combined with Equation (4), the relations of density to diameter and height will be expressed as follows:

$$\rho=\rho_0 \exp(-r_1 D) \ldots\ldots\ldots\ldots (7), \text{ and } \rho=\rho_0 \exp(-r_2 H) \ldots\ldots\ldots\ldots (8)$$

where r_1 and r_2 are constants. These imply that D and H are proportional to the logarithmic ρ/ρ_0, and are proved with the empirical linear relations of ρ—D, and ρ—H in Fig. 3. These equations differ from Yoda's[4] (including the so-called Reineke's equation[11]) where D and H are linearly related to ρ on log-log coordinates in place of semi-log ones.

There existed roughly linear relation between the logarithmic dry weight of aerial part (W_A) and the stand age (t) except for the overmatured, as indicated in Fig. 4 of a previous paper[11]. Combining this relation with Equation (4) the logarithmic equation, $\log W_A=c_0-c \log \rho$ is derived, where c_0 and c are constants.—See the relation of ρ—W_A in Fig. 3. This equation well accords with Yoda's[4], even in the value of constant c (1.5 in Yoda's, and 1.45 in the above).

The relation of total stand basal area (S) to stand age (t) can be formulated through two steps: (1) by means of modification of Equation (5), the individual basal area is expressed as a function of t, i.e.,

$$\pi(D/2)^2=\pi[(d^2/4)t^2-(dd_0/2)t+d_0^2/4]=E_1t^2-E_2t+E_3$$

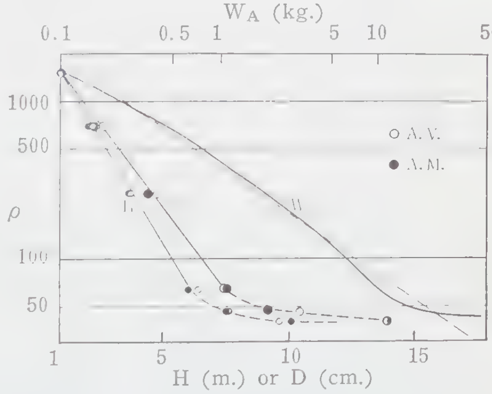

Fig. 3. Relations of tree height (H), diameter (D) and dry weight of aerial part (W_A) to tree density per are (ρ), in standard *Abies* trees (average trees for frequency distribution of D^2) in Forest Unit IV. A. V.=*A. Veitchii*, A. M.=*A. Mariesii*.

where E_1, E_2 and E_3 are constants, and (2) multiplying this equation by Equation (4),

$$S=\rho_0 \exp(-r_0 t)(E_1t^2-E_2t+E_3)$$

The S maximum derived by differentiation should exist at the stand age

$$t=[2E_1+r_0E_2+(4E_1^2+r_0^2E_2^2-4r_0^2E_1E_3)^{1/2}]/2E_1r_0$$

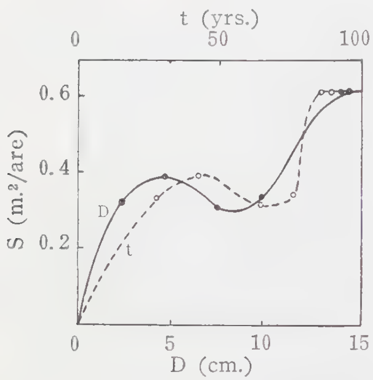

Fig. 4. Relation of total stand basal area (S) to stand age (t) and to diameter (D) of standard trees (average trees for frequency distribution of D²). Forest Unit IV.

In the Forest Unit IV the constants d, d_0 and r_0 are 1.6 mm., 19.3 mm. and 0.075, respectively, so the S maximum is expected to occur at about 40-year-old stand. On the other hand, through modification of Equation (7), S can be expressed as function of D or ρ. For example, as a function of D, $S=\pi\rho_0(D/2)^2\exp(-r_1D)$, and the S maximum exists at $D=2/r_1$.—This diameter size is ca. 42 mm. in this Forest Unit ($r_1=0.048$). In the S-t and S-D curves of Fig. 4 which are formed on the basis of the observed data (Oshima *et al.*[11], Tab. 3), there is a maximum in the first half of both curves, in agreement with the above mentioned results of differentiation. The abrupt increase of S at the beginning of the last half of both curves would be due to the lower mortality in the fully matured stand.

Productive stand structure

The intraspecific competition in stand, as reflected in the difference of weight growth among constituent plants, has to analyzed on the basis of dry matter production. It is necessary, above all, to clarify how the variously sized constituents occupy their position in the productive structure of the stand, because the microenvironmental factors, especially light which influences directly on the dry matter production, are greatly different among stand strata[15),16)]. From this point of view, the productive stand structure of the *Abies* forest was constructed throught the following three procedures. 1) The productive structure of individual trees classified into the dominant, intermediate and suppressed based on tree height, was made up by the modified

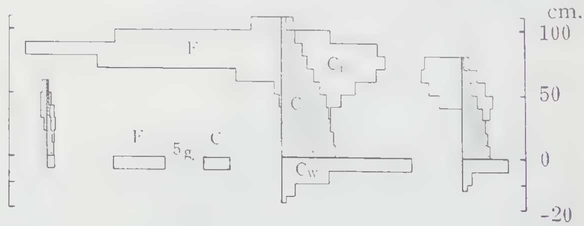

Fig. 5. Productive structures of individual trees of a 20-years-old *Abies* stand in Forest Unit V. The dominant, intermediate and suppressed are classified on the basis of tree height. The symbol F indicates needles; C_B, C_H and C_W stand for branches, trunk and roots, respectively. The areas with these symbols indicate the dry weight of respective organs.

stratifying clip method[7),8),12),17)] (e. g., trees from a 20-year-old stand are shown in Fig. 5). (2) The productive structure of each of three classes was constructed by enlarging their individual structure in proportion to the tree number of each class which was computed from the tree height frequency curve in Fig. 1-A and shown in Fig. 6. (3) The productive stand structure as a whole was reconstructed by means of integration of productive structures of these three classes.

Fig. 6 shows the productive structure diagrams of 14-, 20- and 23-year-old *Abies* stands of Forest Unit V. Also the productive structure of the aerial part of 26-year-old stand gave the same characteristic diagram as the presented stands. In all of these productive structures a larger part of the photosynthetic system, especially in its upper part, was occupied by the dominant, while the non-photosynthetic (trunk, branches and roots) was mainly constituted by those of the dominant together with intermediate class. In case of the 20-year-old stand, the dominant, intermediate and suppressed were the percent. of 66, 27 and 7 in the photosynthetic system, and 52, 41 and 7 in the non-photosynthetic. The stand height and root depth were of course represented by those of the dominant class. The young stand increased with its development in height, canopy height, root depth, total weight of non-photosynthetic system. The total stand needles in dry weight gradually increased with the stand age, and the ratio of non-photosynthetic system to photosynthetic system somewhat increased, too. Because of needle thickness increment with aging of needle, however, leaf area index was kept so constantly (ca. 5.0) that no significant difference was detected among the relative light intensities under these three forest canopies (see Fig. 6).

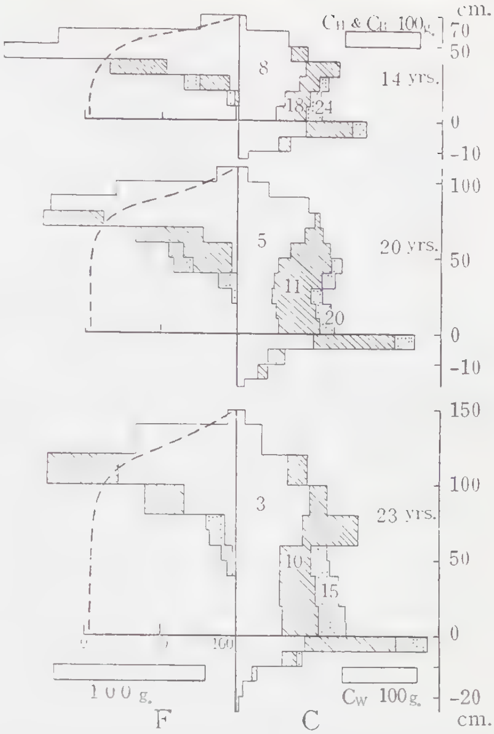

Fig. 6. Productive structures of *Abies* stands, 14-, 20- and 23-years old, resynthesized from individual productive structures of three classes, the dominant (blank), intermediate (hatched) and suppressed (dotted) (cf. Fig. 5). The symbol F stands for photosynthetic system, and C for non-photosynthetic one, including trunk (C_H) and branches (C_B) and roots (C_W). The areas indicate dry weight per sq. m. of respective organs. The figures written in non-photosynthetic system show the tree number per sq. m. in each tree class, and the curves do vertical distribution of relative light intensity.

It will be detailed in a paper being prepared how the difference in productive structure among tree classes causes the different productivity among them.

Summary

In the serially aged *Abies* stands of Forest Units IV and V on Mt. Shimagare, the following were clarified.

1. As for tree height, N- or somewhat L-shaped frequency curves were observed throughout the stand development, while in tree diameter only L-shaped occurred. The frequency distribution of estimated needles weight and tree weight showed extremely L-shaped curves.

2. An average tree for basal area class should be, based on a mathematical theorem, smaller in diameter size, needles weight and tree weight than an average tree for needles weight class and that for tree weight class.

3. Constant annual mortality of *Abies* trees was kept throughout the developmental stages excluding overmatured one. Half of the annual mortality seemed to be given rise to from the defeated trees in the smallest tree class.

4. The trunk diameter and tree height were linearly related to the logarithmic value of tree density, and a linear relation was observed between tree weight and density on a system of log-log coordinates.

5. It was practically as well as theoretically demonstrated that total stand basal area increased gradually in young stage, slightly decreased in mature stage, and again increased rapidly in fully matured stage.

6. Productive structure of *Abies* stands was resynthesized from the productive structure of individual trees classified into the dominant, intermediate and suppressed. The upper part of photosynthetic system of young stand was occupied mostly by the dominants, while in the non-photosynthetic the major parts were constructed by the dominants as well as by the intermediates.

7. With development of young forest stand, the total dry weight of the non-photosynthetic system greatly, and that of the photosynthetic slightly, increased, though the leaf area index (5.0) remained almost constant.

The author desires to express his deep obligation to Prof. M. Monsi and Prof. K. Hogetsu for their valuable advice and suggestions.

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摘　　要

すでに報告された縞枯山のシラビソ，オオシラビソの各林分について，樹木測度の度数分布と立木密度を調べ，さらに若い林分について階級分けされた林分生産構造も調べた。

林分の発達に対して，樹高度数分布は L 型から N 型化する傾向，幹直径分布は L 型が維持される傾向が見られた。個体重と葉重との幹直径に対する関係式から評価されたこれらの重さの度数分布はともに極端な L 型で[illegible]さらに[illegible]葉重，個体重はこれら[illegible]それぞれに対する平均値よりも小さいことが証明された。

過熟木林分をのぞいて，各林分における枯損率は一定（年当り 7.5%）でこの半分は被圧木階級における枯死木で占められた。標準木の幹直径や樹高は半対数式で，地上部重は両対数式で立木密度に対して直線関係を示した。林分の幹断面積合計はその発達とともに単調な増加を示さず，成木林分において少し減少した。

若い林分で得られた生産構造図では，共通して，同化器官の大部分は優勢木階級によって，非同化器官のほとんどが優勢木と中級木との両階級によって占められていた。林分の発達にともなって，非同化器官の林分当りの重さは大いに増大し，同化器官のそれはいくらか増加したが，葉面積指数（5.0）はほとんど変らなかった。

Short Communication

Kinji HOGETSU*, Mitsuru SAKAMOTO** and Hiroshi SUMIKAWA*:

On the High Photosynthetic Activity of *Skeletonema costatum* under the Strong Light Intensity.

宝月欣二*・坂本充**・澄川沢*: 強光下における *Skeletonema costatum* の大きな光合成について

Received September 25, 1959

It is well known that one or several kinds of phytoplankters show remarkable increase in case of the occurrence of red tide in a bay or of water bloom in eutrophic lakes. In every summer the red tide mainly consisted of *Skeletonema costatum* appears along the coast of Haneda in Tokyo Bay. Considering such a rapid increase, we may expect the special features in the dry matter production of concerning alga. For the purpose of analysing the growth, therefore, the authors determined the photosynthetic activity of this alga, and found some interesting facts which are reported in the present paper. Another full detailed paper will be expected in the near future.

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The photosynthesis was measured in the laboratory under varying illumination at 20°-25° within two or three hours after sampling water, and by means of Winkler's method. As the light source 500 and 250 watt reflector lamps were used, and the maximum light intensity employed was about 140 k-lux, which is somewhat stronger than the maximum one in the natural condition. The amount of chlorophyll contained was determined colorimetrically after converting the chlorophyll to pheophytin.

It is remarkable that in the light-photosynthesis curves of *S. costatum* there is no or little depression of photosynthesis even under the condition of the strongest light intensity, in opposition to the results in many former reports concerning the photosynthesis of phytoplankters. The assimilation number measured falls in an extremely high range of 24-45 O_2 mg./chlorophyll mg./hr. These high values have not been reported upon the natural algal population so far as we know (cf. Table). Also *Microcystis aeroginosa* which is a main component of water bloom shows the same photosynthetic behavior in its developing stage.

Assimilation number (O_2 mg./chl. mg./hr.) of *Skeletonema costatum*, the main component of the red tide in Tokyo Bay, and of several algal populations in some Japanese lakes and sea.

Location	Date	Assimilation number	Dominant species	Investigator
Tokyo Bay	26 VI 1958	24.1	*Skeletonema*	Hogetsu, Sakamoto & Sumikawa
	10 VII 1958	45.2	,,	,,
	10 VII 1958	28.9	,,	,,
	30 VIII 1958	27.1	,,	,,
	26 VI 1959	28.2	,,	,,
Pond in	6 VIII 1958	12.7	*Microcystis*	Hogetsu, Sakamoto & Sumikawa
Park Himonya	23 VII 1959	33.2	,,	,,
(Meguro, Tokyo)	31 VII 1959	22.7	,,	,,
	15 VIII 1959	21.1	,,	,,
	25 VIII 1959	11.0	,,	,,
Lake Kasumigaura	16 V 1957	9.1	*Microcystis*	Ichimura & Aruga, 1958
Lake Jônuma	29 X 1956	8.2	*Chlamidomonas*	,,
Lake Tega	29 V 1956	6.8	*Fragilaria*	,,
Lake Suwa	6-8 XI 1950	6.3		Hogetsu & Ichimura, 1954
Lake Haruna	29 X 1956	5.7	*Asterionella*	Ichimura & Aruga, 1958
Lake Kawaguchi	28 VI 1956	4.6	*Melosira*	,,
Lake Ashinoko	23 V 1958	1.4	,,	,,
The Pacific Ocean off Japan	2-9 V 1958	1.24-9.1*		Ichimura & Saijô, 1959

* These values are computed by the authors from Ichimura & Saijô's data obtained by the C^{14} method.

抄　　　録

○大麦子葉鞘細胞壁からの Ca の除去

Carr, D. J. and Ng, E. K., The sequestration of calcium from preparations of wheat coleoptile cell walls. Physiol. Plantarum **12**: 264–274 (1959).

○細胞伸長におけるキレート剤とオーキシンの活性に対するpHの影響

Ng, E. K. and Carr, D. J., Effects of pH on the activity of chelating agents and auxins in cell extension. ibid. **12**: 275–287 (1959).

キレート剤が子葉鞘の伸長を促進することから，オーキシンはキレート剤のように細胞壁中の Ca-pectate からCa を除いて細胞壁の loosening をもたらすという仮説が最近提唱されている。これに対してキレート剤の作用は pH と密接な関係をもつことから、EDTA などの Ca キレート作用や伸長促進作用を pH の関係において検討した[illegible]

[illegible]，その Ca [illegible]に対する EDTA の影響をクエン酸燐酸緩衝液を用い pH 5 および 7 でみている。その結果、緩衝液だけで pH 5 の場合いちじるしく細胞壁中の Ca が除かれ，EDTA は Ca をキレートせぬだけでなく緩衝液の作用を阻害する。このことから EDTA による伸長促進作用は Ca 除去によるものではないと結論している。

後者ではさらに DIECA, 8-HQ, EDTA, DTCの大麦及び燕麦子葉鞘の伸長に対する作用をみている。EDTA は酸性側では Ca をキレートしないにもかかわらず伸長を促進し，pH 6.5 よりアルカリ側では伸長を促進しない。したがって EDTA による伸長促進作用はその Ca キレート作用によるものでないと結論し，DIECA, 8-HQ, DTC を用いた実験からも Ca キレートによる伸長促進機構を否定している。

オーキシン作用機構に関しては上述の仮説を完全に否定はしていないが，キレート剤の伸長促進機構には細胞壁中の Ca の変動は関係せず，むしろ作用群に Cu 或は Fe をもつインドール酢酸酸化酵素の阻害剤として働き，間接に子葉鞘の伸長を促進するものと考えている。

（増田芳雄）

リ ボ 核 酸 の 核 由 来

M. Zalokar, Nuclear Origin of Ribonucleic Acid. Nature **183**: 1330 (1959).

RNA がどこで造られるかの問題についてはすでに[illegible]するものであるという[illegible]されてはいたが，必ずしもまだ[illegible]なかった。

Neurospora crassa の生の菌糸を遠心するとその[illegible]，エルガストプラズム[illegible]，核，細胞質[illegible]各層にわけられ，各分画を細胞化学反応によって調べ，細胞質中 RNA [illegible]プラズムに，若干が[illegible]コンドリアに，そして[illegible]にはまったく見られず核には比較的少ないことなどが知られた。標識前駆物質（Uridine-5, 6-^{3}H, 640mc. per m. mole, 1000μc./ml.）を与えて遠心して固定, 0.5%冷トリクロール酢酸で洗って RNAに結合したものだけを残してオートラジオグラフにとってそれがどこにとりこまれているかを見た。与えた時間が 1～4 分では標識は核分画だけに現われ，4 分以後は次第に細胞質内 RNA に現われ，1 時間ではエルガストプラズムにきわめて強くなり，ミトコンドリアにも活性が見られた。しかし上澄には不活性のままであって，結局標識分布は各分画における RNA の比較量におおよそ平行的であり，エルガストプラズムにおける標[illegible]は移りそれ[illegible]につれて増大するのが明らかであった。

これらの実験結果は明らかに細胞内の RNA が核において造られ、すなわち遺伝子による直接の産物であって、これから細胞質中へ移動してゆくものであることを示しており、従来多くは推測にとどまっていた RNA 核起原の仮説に直接的な[illegible]を示唆するものである。

（[illegible]）

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（例） Ryan, F. J., and Beadle, G. W., Amer J. Bot. 30: 784 (1943).

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Leaf Development Following Longitudinal Split of the Shoot Apices of the Germinating Embryo and the Seedling in *Sesamum indicum* L.

by Jun HANAWA*

[illegible]

Received June 5, 1959

It was observed by the present author[1),2)] that when the apical meristem of the embryo of *Sesamum indicum* L. was split longitudinally into two halves along the intercotyledonary plane, each of halves regenerated a new shoot, which was furnished with a double-leaf formed by lateral fusion of the opposite first leaves. In the present investigation, longitudinal incisions were made on the shoot apices of germinating embryos and young seedlings, for the purpose of studying the effects of such operations at different developmental stages upon the first leaf development.

Longitudinal incisions on the shoot apices were made every one day during the period of four days after sowing. The techniques of incision and the method of raising the operated seedlings described in the previous papers[1),2)] were followed. At the same time, observations were made on the growth of intact shoot apices and leaf primordia in order to relate the effects of the operation with the stages of development.

Development of the Shoot Apex and Leaf Primordium of the Unoperated Seedling

It is appropriate to mention here briefly some aspects of the development of the apical meristem and leaf primordium of the unoperated seedling, in order to check the effects of the operation with the stages of development. Detailed descriptions of the growth of the shoot apex and the leaf formation will be reported elsewhere.

On the shoot apex of the mature embryo, two buttresses of the first leaves are situated on both shoulders of the epicotyl, and the rather concave shoot apex proper lies between them. Although the tunica stratification has not yet fully developed, two layers are discernible. In the second layer are found periclinal walls, which somewhat obscure the stratification. The corpus, which is about three cells in depth, is followed by a rib-meristem, the cells of which are filled with granular reserve materials (Fig. 1A). The distinction between the shoot apex proper and the foliar buttresses is rather difficult. Procambia, differentiated in the hypocotyl axis

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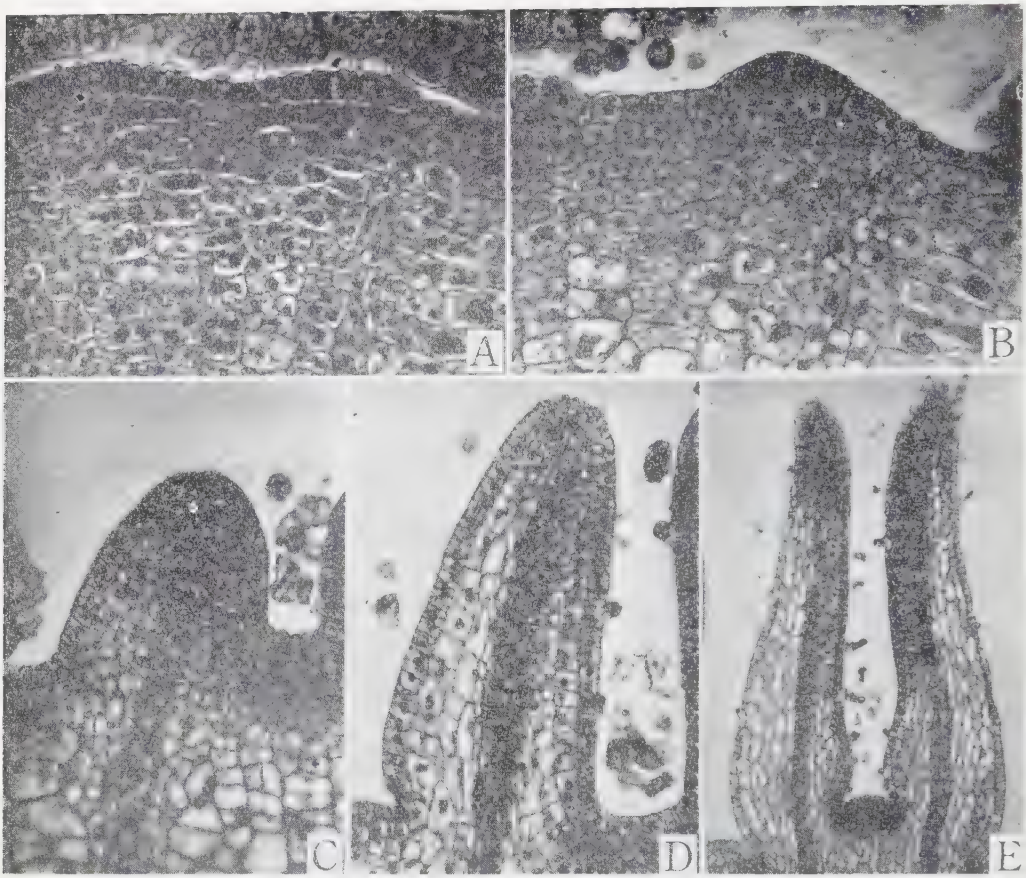

Fig. 1A–E. Shoot apex and the first leaf primordium. A, shoot apex of a full-grown embryo, with two foliar buttresses. B, shoot apex of a one-day seedling. C, D and E, the first leaf primordium two days, three days and four days after sowing respectively. A and B, ×240; C and D, ×220; E, ×80.

during later stages of embryogeny, extend up to the fourth layer from the surface of the buttress. The apical meristem is, so to speak, in a juvenile state at this developmental stage. It develops into an adult shoot apex with a characteristic structure after four to five days of growth.

Within a period of one day after sowing, cell divisions occur in the foliar buttresses as well as in the shoot apex proper (Fig. 1B). The activity of cell division is higher in the former than in the latter. The stratification of the apical meristem becomes rather less distinct than in the resting embryo on account of divisions in the sub-surface layer of the foliar buttresses. Granular reserve materials diminish from the cells of the rib-meristem, which then begin to vacuolate. In general, the cells of the apical meristem of the one-day seedling are somewhat larger and stain more evenly than those of the resting embryo (Fig. 1B).

One day after sowing, the first leaf primordia are somewhat elevated by anticlinal divisions in the surface layer and divisions in various directions in the corpus (Fig. 1B). Beneath the foliar buttress the procambium starts acropetal differentia-

tion and enters the meristematic region of the leaf base, reaching four to five cells beneath the tip of the primordium.

Two days after sowing the primordium attains a height of about 75 microns (Fig. 1C). It grows, at this stage, by the apical growth, which results from the activity of a subapical initial. The upper half of the primordium keeps a meristematic appearance, and in the lower half there occurs no intercalary growth yet. The procambium reaches about 2/3 height of the primordium, that is, four to five cells beneath the tip.

Three days after sowing, the primordium attains a height of 200 to 250 microns (Fig. 1D). At this stage the apical growth activity begins to decline and is followed by an intercalary growth. The vacuolation of cells proceeds acropetally from the basal portion. Only the procambium and the two strips of meristematic cells located along the both margins of the leaf axis stain deeply.

Four days after sowing, the primordium is 600 to 700 microns high (Fig. 1E). The cells are elongated except those of the apical region. The cells of the adaxial side are less elongated and less vacuolated than those of the abaxial side. In the lower adaxial portion, there appears an adaxial meristem which increases the thickness of the leaf axis. By the activity of the marginal meristem, which has been organized by this stage, the extension of lamina is going on.

Effects of Operations

In the case of the pre-germination operation, as the author[1),2)] previously reported, the opposite leaves of the first pair were fused into a double-leaf when a regeneration of the apical meristem took place. However, in the post-germination operation which was made in the present study, the first opposite leaves were not transformed into a double leaf, but remained opposite as normal. When the incision fell successfully in the median plane, two regenerated shoots were equal in size and the first opposite leaves on one shoot were symmetrical in shape to those on the other shoot in reference to the plane of incision (Fig. 2A, B). Since the first leaves remained opposite, the subsequent leaves were not disarranged, maintaining the decussate phyllotaxis (Fig. 3A, B). This applies to all the cases irrespective of the time of the operation. Thus it is evident that the regeneration ability of the apical meristem of the dormant embryo is kept also in the growing shoot at any developmental stage.

On the contrary, the morphology of the first leaves which developed after the operation offered interesting changes relating to the time of the operation. When the operation was made one day after sowing, the first leaf primordia grew normally, developing nearly symmetrical laminas (Fig. 2C; Fig. 4A-C). Only in the basal portion of the leaf blade, the recovery of the lamina was slightly incomplete, a small basal area on the cut side being defective. When the operation was made two or three days after sowing, the deficient area of the lamina on the cut side more extends acropetally, and the symmetrical lamina was developed in the distal half (Fig. 4D-F). When the operation was made on the shoot apex of the four-day seedling, the leaf shape was utterly odd, the half of the lamina on the cut side

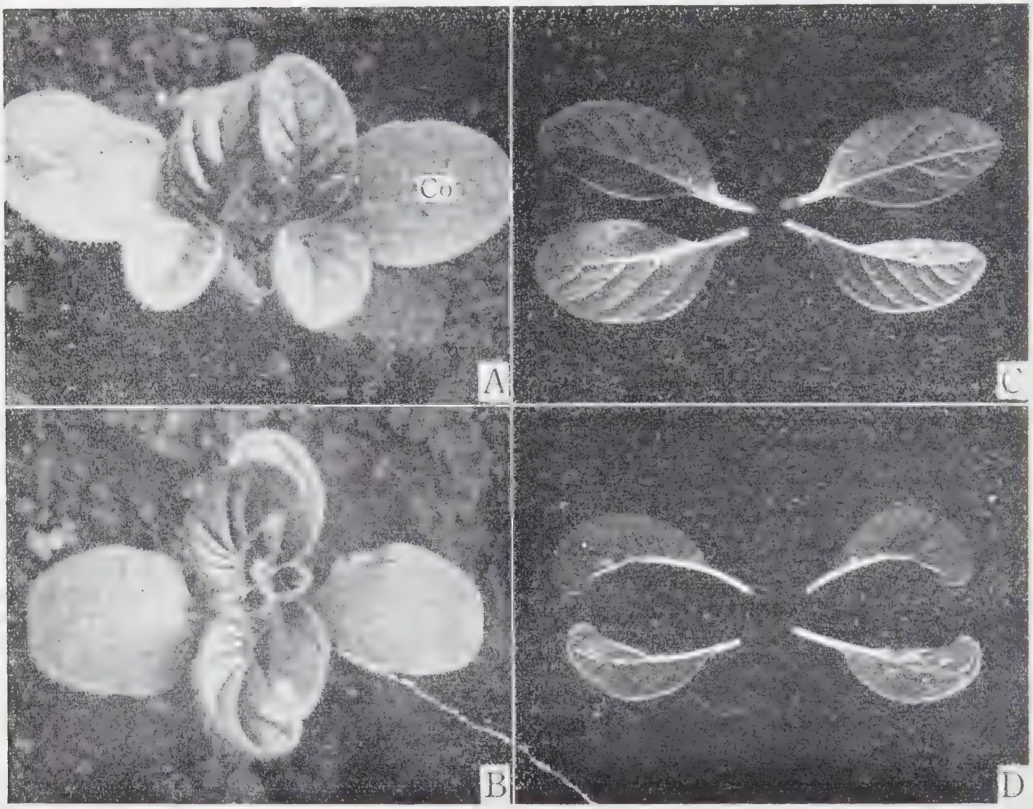

Fig. 2A-D. A and B, young twin plants, grown after splitting of the shoot apex of a one-day and a four-day seedling respectively. C and D, final shape of the first leaves of the plant A and B respectively. Co: cotyledon. ca. ×2.

being deficient (Figs. 2D; 4G-I). The results above mentioned indicate that the potency of laminal development is fully retained in the apical region of the leaf primordium, whereas in the basal part the potency has already been restricted or lost early during the leaf development. The decrease of the potency accompanying the growth of the leaf primordium is suggested also by the incompletely organized vascular system of the midrib. On the cut side, the branch bundle from the median bundle was occasionally not differentiated or, if present, it was thinner than that on the other side. The branch occurred in some case at a higher level than in normal (in the latter it occurs at the leaf insertion). Fig. 3 shows that, when the primordia were split one day after sowing, vascular bundles of the midrib were almost normally differentiated, except for that in one of the leaves one of the branch bundles was absent (B, upper side), and in another one the branching of the bundle on the cut side took place at a higher level than on the other side (A, lower side). As the time of the operation is delayed the differentiation of the branch bundle is more inhibited. However, it seems to be noteworthy that the vascular bundle is differentiated deep in the center of the midrib, although the procambium must have been laid bare on the cut surface when the leaf primordium was split by the

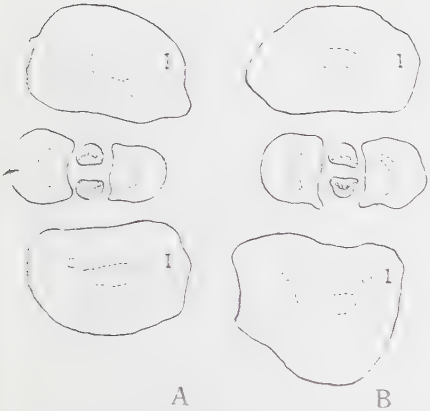

Fig. 3. Transections through the apices of the regenerated shoots from halves of the apical meristem, operated one day after sowing. A, the one shoot of the twin plant. B. the other one. I: the first leaf. ×28.

Fig. 4. Transections at right angles to the midrib of the mature first leaves, which developed after the operation. Sections are taken from three points: petiole, lower portion and the broadest portion of the blade. Groups A-C, D-F, and G-I, are the sections of these three points of the leaves on the shoots operated one day, three days and four days after sowing respectively. ×14.

operation. This fact leads to a conclusion that, when the procambium is exposed by incision, it can no longer develop into the vascular tissue, or be transformed into the marginal meristem, but it differentiates into the cortical parenchyma or epidermis.

Discussion

A number of investigations have been made by various authors on the problems of apical growth in vascular plants with the method of longitudinal incision of the shoot apex. These investigations have revealed a conspicuous developmental potency and a self-determining nature of the shoot apex, and presented experimental explanations of the determination of leaves[3),4)]. However, in these experiments the double-leaf formation in plants with decussate phyllotaxis, as it was the case in the pre-germination embryo of *Sesamum*[2)], has not yet been recorded. Soma[5)] has reported that no double-leaf was induced by the orthogonal or diagonal split of the shoot apex in *Euphorbia lathyris*, a plant with decussate leaves. In *Sesamum* also, as stated above, the operation of the shoot apex more than one day old induced no double-leaf, but it resulted in the formation of the normally situated first opposite leaves instead. Unions between the successive leaves by means of operation were observed in some case; for instance, Snow and Snow[6)] for *Lupinus*, and Snow and Snow[7)] for *Epilobium* found the fusion occurred between P_1 and P_2, or I_2 and I_3.

These facts suggest that the formation of the double-leaf is a distinctive re-

sponse of the shoot apex of the resting embryo of *Sesamum* to the longitudinal incision, and is caused by certain characteristic features of such an apex. The author, in a previous paper[2], has assumed a process of the fusion of leaf primordia in the embryo of *Sesamum*; such a process must depend on the narrowness of the shoot apex, where opposite leaf primordia are situated close to each other. The formation of the double-leaf, i. e., the fusion of two leaf primordia, by the incision, is restricted to the buttress stage of leaf development. Thus, another cause probably lies in that in the shoot apex of the pre-germination embryo the delimitation between the shoot apex proper and the foliar buttresses is not clear. This can be interpreted as an expression of the intrinsic unity of the leaf and the stem. A comparable case was observed in *Bryophyllum* by Gavaudan[8]; whether the first pair of leaves of the buds treated with 2, 4-D or 1, 3, 5-TIBA gives rise to a gamophyll by their fusion, or to a pair of normal leaves, depends upon the stage of development attained by the bud at the time of treatment.

Then, what are the factors that prevent the fusion of leaf primordia following the operation of the one-day seedling? At least two conditions may be pointed out: (1) the increasing distinction between the shoot apex and the leaf primordium and (2) the differentiation of the procambium in the leaf primordium. These two points are the expressions of the advance of the leaf development. Because of these conditions the process of the fusion of leaf primordia, such as was assumed in the previous paper[2], may be obstructed.

The defective leaf blade resulting from incision is found to be closely related to the developmental stage of the leaf primordium at the time of operation. The more advances the differentiation of the primordium under the operation, the less the cut half of the blade is recovered. According to Esau (1953, p. 443)[9], the development of the leaf may be divided into three stages: (1) formation of the foliar buttress, (2) formation of the leaf axis, and (3) formation of the lamina. For the first leaves of *Sesamum*, these stages may correspond respectively to those of the primordia of the one-day, two to three-day, and four-day seedlings. At the first stage, the cells of the foliar buttress are fully meristematic and the half of the buttress is capable of producing a complete leaf. When the erection of the leaf axis begins at the second stage and the meristematic cells are confined to the apical region of the primordium, a defective area appears in the lower portion of the blade. The deficient part suggests that the marginal meristem was not reorganized on that portion. If the primordium is split prior to the cessation of apical growth, the marginal meristem may be organized on the wounded side in the course of apical growth. After the apical growth has come to an end, and it has been followed by the intercalary growth and laminal extension, the split primordium fails to reorganize the marginal meristem.

Summary

Effects of the longitudinal split of the shoot apices of the germinating embryo and the seedling in *Sesamum indicum* L. upon the development of the first leaf were studied. The incisions were made in the plane running through the first leaf

primordia. When the operation was made one day after sowing, the first leaves were almost normal both in shape and position, double-leaf being not formed. Following the operation made two to four days after sowing, the first leaf developed into an odd shape defective of lamina on the cut side. Comparing the leaf blade deficiency caused by the operation with the stages of development of the leaf primordia at the time of operation, the following are evident: when the leaf primordium of the buttress stage is split into two halves, both of them are capable of developing complete leaves; the leaf primordium under apical growth is still partially capable of developing leaf blade when it is split; but after the apical growth is over and the laminal development has begun, the half of the split leaf primordium is no longer potent of blade formation on the cut side. The decrease of the potency accompanying the growth of the leaf primordium is suggested also by the incompletely organized vascular system of the midrib. When the time of operation was delayed the differentiation of the branch bundle on the cut side from the median bundle was omitted. After the operation the vascular bundle was differentiated deep in the center of the midrib, although the procambium must have been laid bare on the cut surface when the leaf primordium was split by the operation.

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摘　　　要

こ[illegible]する第一葉原基を縦の面で縦に切る手術を行ない，それが第一葉の形成に及ぼす効果をみた。手術を種まきの 1 日後に行なうと，原基の半分から生じた第一葉はほとんど正常な形態をもち，位置も対生のままであった。これは種まき前に手術を行なうと第一葉が対生の位置から互いに接近して双生葉となる[1),2)]ことと対照的である。この差異は種まき後 24 時間内に，頂端分裂組織の[illegible]が変化して，手術に対する反応が異なってくるためであろう。種まきから 2 日以後の手術では，第一葉は[illegible]で葉身の片側に欠損ができる。手術を行なう時期がおそくなるにつれて，葉身の基部から先端[illegible]。4 日後の手術では葉身の片側が全くつくられない。葉の発育段階と，手術による葉身の欠損とを対照してみると次のことが明らかである，すなわち，葉原基は，buttress の時期には，二分されても完全な葉を形成する能力がある；葉原基は，頂端生長を続けている時期には，二分されても，葉の先端部にはなお[illegible]葉身を展開することができる；しかし頂端生長が終り，辺縁分裂組織の活動が始まったのちは，縦に二分された葉原基は，葉身の片側を形成する能力を失なう。葉原基の生長に伴なう形成能力の減少は，中肋の維管束系の構成の不完全さにも見られる。すなわち手術の時期がおそくなるにつれて主脈からの側脈は省略されてしまう。しかし，葉原基が縦に二分されたときは，前形成層は切断面に露出していた筈であるが，後に維管束は中肋の中心部に分化している。それゆえ，前形成層は皮層や表皮の組織を形成する能力があると考えられる。

Biochemical and Genetical Studies on Anthocyanins in Eggplant*

by Yukihide ABE** and Kanji GOTOH**

阿部幸穎**・後藤寛治**： ナスのアントシアニン色素およびその遺伝

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The fact that the acylation of anthocyanin takes place in the presence of dominant allele was first demonstrated by Scott-Moncrieff[1] in several genotypes of *Papaver rhoeas* on the basis of pigment analysis according to the Robinsons. Harborne[2),3)] found in his experiments on potato plants that at least four biochemical characters, i. e. acylation, hydroxylation, methylation and glycosidation of anthocyanins are likely controlled by a single pair of genes. On the other hand, the present authors have described in their preliminary reports[4),5)] that in eggplant the acylation as well as glycosidation seems to be controlled by a single pair of genes. The present paper deals with this subject in detail.

Experimental

1) *Plant materials:* Two horticultural varieties of eggplant (*Solanum melongena* L.), Burma and Black Beauty, their F_1 hybrids and F_2 plants were used for the present study. Besides, anthocyanins of the related horticultural varieties, Sendai-naga No. 1, Shin-kuro Heta-murasaki and Emerald, were also examined. All of the materials have been maintained by successive self-pollination during recent five years.

2) *Pigment analysis:* Paper-chromatographic analyses were carried out, chiefly after Bate-Smith[6),7)], Abe and Hayashi[8)], according to the ascending method using the following solvent mixtures on Tôyô No. 50 filter paper:

AA. H (5, 1, 5)..............AcOH/36% HCl/H_2O (5:1:5, v/v),
AA. H (3, 1, 8).............. ,, / ,, ,, / ,, (3:1:8, ,,),
Pro. H ....*iso*-Propanol/5% HCl (55:45, ,,),
iso A. H (21, 5, 4) ....*iso*-AmOH/36% HCl/H_2O (21:5:4, ,,),
Bu. A ....*n*-BuOH/AcOH/H_2O (4:1:5, ,,),
Bu. H ....*n*-BuOH/36% HCl/H_2O (7:2:5, ,,),
Cr. A ....*m*-Cresol/AcOH/H_2O (50:2:48, ,,),
Phen........................Phenol/H_2O (9:1, in weight).

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For the identification of individual pigments, the authentic specimens of corresponding anthocyanins were always run in parallel, which were kindly supplied by Prof. K. Hayashi.

3) *Examination of anthocyanins:* Full-colored fruit-coats, petals, and epidermal and cortical parts of the stem were analyzed with six plant varieties mentioned above. Each colored portion (3～5 g.) was throughly extracted with 1% methanolic hydrochloric acid (2 ml. per g. of fresh material). The filtered extract was added with an equal volume of 20% hydrochloric acid and heated at 95° for 40 minutes. After dilution with water, the anthocyanidin produced was extracted with minimum volume of amyl alcohol and transfered into dilute hydrochloric acid by the addition of petroleum ether and subjected to the paper-chromatographic tests using three solvent mixtures, AA. H (5, 1, 5), Pro. H and iso A. H. In any of the varieties examined, delphinidin was detected together with a trace of cyanidin. In these solvent mixtures, Rf values were 0.22, 0.25 and 0.28 for delphinidin and 0.34, 0.34 and 0.63 for cyanidin, respectively. In order to clarify the glycoside nature, crude extracts were directly chromatographed using the solvent mixtures, isoA. H (21, 5, 4), AA. H (3, 1, 8), Bu. A, Bu. H and Cr. A, respectively.

In genetic experiments, pigment compositions of the parental varieties, Burma and Black Beauty, F_1 hybrids and F_2 plants were analyzed in a similar manner. Besides, fruit-coat-extracts of the parent and F_1 plants were examined by two-way paper-chromatography, using AA. H (3, 1, 8) in one direction and Bu. H in another.

4) *Main anthocyanins of Burma and Black Beauty:* Cold 1% methanolic hydrochloric acid extracts of the fruit-coats of Burma and Black Beauty were prepared and subjected to a large scale paper-chromatography using the solvent, Bu. H. The anthocyanin band was separated by cutting and eluted with 1% methanolic hydrochloric acid in a manner similar to the descending method. The eluate was then concentrated *in vacuo* over sodium hydroxide. The anthocyanin was purified further by repeated precipitation from its acidified ethanolic solution by the addition of ether and analyzed paper-chromatographically. On the other hand, the anthocyanin, which was obtainable as picrate in hair fine needles (m. p. 179～180°) from the fruit-coats of Burma, was also subjected to the paper-chromatographic and spectrophotometric examinations. The purified fractions obtained were hydrolyzed by boiling in 20% hydrochloric acid for 3 minutes, and the sugar-free pigments were examined by paper-chromatography. Rf values of aglycones derived from Burma- and Black Beauty-glycoside were quite identical with each other and also with the authentic delphinidin.

The measurement of absorption maxima was made on purified preparation of the glycosides and of the aglycones derived therefrom by the usual way. The results are shown in Table 1, from which it can be seen that the aglycones from both plant sources are identical with delphinidin and that the Black Beauty glycoside agrees with delphinidin 3-glucorhamnoside newly isolated from a variety of *Tulipa gesneriana* by Shibata[9].

As regards paper-chromatographic behavior in several solvent mixtures, it is indicated that Burma-glycoside agrees with nasunin, and Black Beauty glycoside

Table 1. Absorption maxima of Burma- and Black Beauty-anthocyanin.

Pigment in 0.1% ethanolic hydrochloric acid	Absorption maxima (mμ)			
Burma-glycoside	550	310	281	225
Black Beauty-glycoside	552	355	280	222
Delphinidin 3-glucorhamnoside	552	355	279	219
Burma-aglycone	556	357	280	225
Black Beauty-aglycone	555	357	280	225
Delphinidin	555	358	278	222

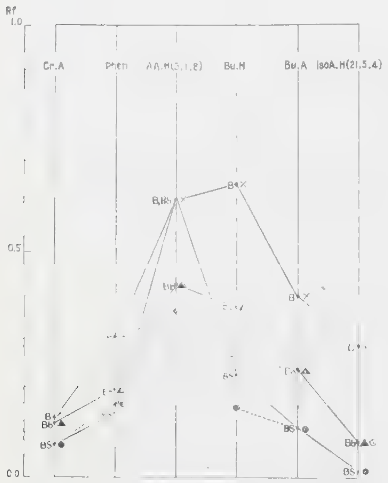

Fig. 1. Rf values of Black Beauty- and Burma-anthocyanin before and after saponification (Tôyô No. 50 Filter Paper at 25±2°). B: Burma-anthocyanin, Bb: Black Beauty-anthocyanin before and after saponification, BS: Burma-anthocyanin after saponification, ○: delphinidin 3-monoglucoside, ▲: delphinidin 3-glucorhamnoside, ●: delphinidin 3, 5-diglucoside, ×: nasunin (*p*-hydroxycinnamoyl delphinidin 3-diglucoside).

with delphinidin 3-glucorhamnoside, respectively, as shown in Fig. 1.

The presence or absence of acyl group in these anthocyanins was shown by the following way. Each glycoside was treated with 10% aqueous sodium hydroxide for 40 minutes at room temperature in an atomosphere of hydrogen. After slight acidification, the solution was paper-chromatographed in a large scale using the solvent, AA. H (3, 1, 8). The pigment band formed was cut off and eluted as usual, and re-chromatographed with the solvent, Bu. H, and treated as above. With these pigment concentrates, paper-chromatographic comparison was made under precaution. In the case of Black Beauty-glycoside, no difference was observed in Rf values before and after saponification. However, the Rf values of saponified Burma-glycoside was higher than that of delphinidin 3, 5-dimonoglucoside in two solvent mixtures, Bu. H and AA. H (3, 1, 8), respectively, but was nearly equal in other solvent systems. This relationship may be comparable to that found between delphinidin 3-monoglucoside and delphinidin 3-glucorhamnoside.

An acidified solution obtained after saponification was thoroughly extracted with ethyl ether and the etheral solution was evaporated. A colorless crystalline residue was obtained in the case of Burma-anthocyanin. This substance was identified as *p*-hydroxycinnamic acid by means of paper-chromatography[10] and color reaction, as shown in Table 2.

Further information about the nature of these glycosides could be obtained by partial hydrolysis[8]. A solution of the glycoside in 10% hydrochloric acid was heated at 70°. During the course of hydrolysis, small, definite amount of solution was pipetted out at definite intervals and paper-chromatographed using the solvents, Bu. A, Bu. H and AA. H (3, 1, 8). The results have shown that hydrolysis of each

Table 2. Rf values of acidic components in Burma-anthocyanin. One-dimensional ascending chromatography at $25 \pm 2°$; graysh blue color develops with Bray's diazotyzed *p*-nitroaniline[10].

Solvent mixture for irrigation	*p*-Hydroxycinnamic acid	Organic acid from Burma-anthocyanin
Benzene/AcOH/H_2O (2:2:1, v/v)	0.50	0.50
m-Cresol/AcOH/H_2O (50:2:48, „)	0.82	0.82
n-BuOH satd. with 5 N NH_4OH aq.	0.16	0.16
n-BuOH/AcOH/H_2O (4:1:5, v/v)	At solvent front	At solvent front

glycoside proceeds according to the following scheme:

Black Beauty-anthocyanin:

Del* 3-glucorhamnoside→Del 3-glucoside→Del.

Burma-anthocyanin:

Burma-anthocyanin→Del diglucoside+rhamnose—↗Del 3-glucoside→Del. ↘Del 5-glucoside→Del.

Burma-anthocyanin after saponification:

Del diglucoside+rhamnose→Del diglucoside—↗Del 3-glucoside→Del. ↘Del 5-glucoside→Del.

*Del: delphinidin.

The chromatograms obtained in the case of saponified Burma-anthocyanin are illustrated in Fig. 2.

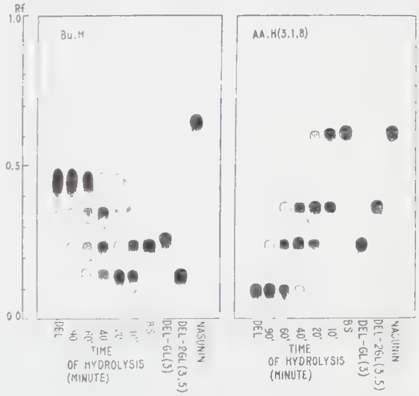

Fig. 2. Paper-chromatograms of the products of partial hydrolysis, which were derived from saponified Burma-anthocyanin. BS: saponified Burma-anthocyanin, DEL: delphinidin, DEL-Gl (3): delphinidin 3-monoglucoside, DEL-2Gl (3, 5): delphinidin 3, 5-diglucoside, *: delphinidin 5-monoglucoside.

Results and Discussion

Paper-chromatographic examinations of the crude extracts have shown that five varieties belonging to a group of small-fruited eggplant, e.g., Burma, Sendai-naga No. 1, Heta-murasaki, Shin-kuro and Emerald, contain an acylated delphinidin diglycoside as the main pigment component. The Rf values of this pigment agree with those of nasunin (*p*-hydroxycinnamoyl delphinidin 3-diglucoside) which was previously isolated from certain variety of eggplant by Kuroda *et al.*[11] On the other hand, Black Beauty belonging to a group of large-fruited eggplant contains non-acylated glycoside. In all of these varieties, other types of anthocyanins also present in minute amount. Within a single variety, however, the anthocyanin composition of stem and flower is identical with that of the fruit-coat in general.

From the results of the examination of main anthocyanins in fruit-coats of

Burma and Black Beauty, it follows that the Black Beauty-anthocyanin is nothing but delphinidin 3-glucorhamnoside, and the Burma-anthocyanin is different from nasunin. It might be concluded that Burma-pigment is *p*-hydroxycinnamoyl derivative of delphinidin 3, 5-diglucoside, which is further combined with rhamnose (probably one molecule).

Next, the anthocyanin components of parent plants, F_1 hybrids (stem, flower and fruit-coat) and F_2 progenies (stem and fruit-coat) obtained from the cross between Burma and Black Beauty (using the latter as pollen parent) were examined in detail. The color* of the fruit-coats of Burma, F_1 and F_2 plants containing Burma-anthocyanin is deep purple to deep reddish purple, and that of Black beauty and F_2 plants having Black Beauty-anthocyanin is dark brownish purple. The anthocyanin composition of F_1 hybrids was found to be identical with that of Burma, although some other anthocyanins were also present in minute amount. Accordingly, the Burma-type of anthocyanin proved to be completely dominant over the Black Beauty-type, as shown in Fig. 3. In F_2 plants, 76 individuals had anthocyanin composition of the Burma-type and 22 showed that of the Black Beauty-type. Intermediate type could not be found. In consequence, simple Mendelian segregation ratio ($x^2=0.4301$, $P=0.7\sim0.5$) was obtained.

Fig. 3. Paper-chromatograms showing the anthocyanin composition of fruit-coats in F_1 and F_2 plants obtained from the cross, Burma (B) × Black Beauty (BB). Left being developed by AA. H (3, 1, 8); right by Bu. H, respectively.

The tests of saponification and partial hydrolysis have also confirmed that the main pigment in fruit-coats not only of F_1 plants but also of F_2 plants of Burma-type was Burma-anthocyanin itself.

Table. 3. Rf values of sugar components in Burma- and Black Beauty-anthocyanin. Chromatogram was obtained by one-dimensional ascending method at room temperature, and spot was developed with aniline hydrochloride reagent[13].

Sugar	Bu. A	Phen
Sugar from Burma-anthocyanin A	0.41	0.68
〃 B	0.21	0.38
Sugar from Black Beauty-anthocyanin A	0.40	0.67
〃 B	0.19	0.38
Xylose	0.32	0.52
Rhamnose	0.42	0.68
Galactose	0.22	0.44
Glucose	0.23	0.39

* The color was determined according to "Guide to Color Standard" (Tokyo, 1951).

From these observations, it follows that the acylation and glycosidation, by which Burma- and Black Beauty-anthocyanins may be distinguished from each other, are controlled by a single pair of genes. It should be noted that the anthocyanin composition of the stem is identical with that of fruit-coats in F_1, F_2 and parent plants, respectively.

The authors' thanks are due to Prof. Dr. K. Hayashi (Botanical Institute, Faculty of Science, Tokyo University of Education) for his valuable suggestion and kind supply of several anthocyanin specimens.

Summary

Paper-chromatographic analysis of anthocyanin occurring in flower, stem and fruit-coat of eggplant showed that Burma, Sendai-naga No. 1, Shin-kuro, Heta-murasaki and Emerald contain an acylated anthocyanin, whose Rf values agreed with nasunin, whereas Black Beauty contains delphinidin 3-glucorhamnoside as major component. Within a single variety, the main anthocyanin proved to be the same one in stem, flower and fruit-coat.

The complex anthocyanin of Burma-type was shown to be *p*-hydroxycinnamoyl derivative of delphinidin 3, 5-diglucoside combined with rhamnose.

In the crossing experiment, it was observed that the F_1 hybrid between Burma and Black Beauty produced an acylated anthocyanin of Burma-type and in F_2 generation Burma- and Black beauty-type segregated in accordance with the simple Mendelian ratio of 3:1. Accordingly, two biochemical characters, i. e. the acylation and glycosidation in anthocyanin molecule are shown to be controlled by a single pair of genes.

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摘　　要

ナス 6 品種の花・茎および果皮のアントシアン色素をペーパー・クロマトグラフイで調べた結果，ビルマ，仙台長 1 号，真墨，薄紫およびエメラルドの主色素は Nasunin に Rf 値が一致し，ブラツク・ビユーテイーの主色素は Delphinidin 3-glucorhamnoside と一致した。また，ビルマから分離した主色素は *p*-hydroxycinnamoyldelphinidin 3, 5-diglucoside に更に rhamnose の結合したものであることが判つた。

ビルマ×ブラツク・ビユーテイーの雑種第 2 代までの植物について茎および果皮の色素を調べたところ，同一個体内では果皮と茎との主色素は同一であり，F_1 植物はビルマ型組成をとり，F_2 植物ではビルマ型組成をもつものとブラツク・ビユーテイー型組成を示すものとが 3:1 の比で分離した。したがつて，両色素の間の acylation と glycosidation に関する差異は一対の対立遺伝子の作用によると考えられる。

The Occurrence of Gibberellins in Mature Dry Seeds

by Yutaka MURAKAMI*

村上　浩*：完熟種子におけるジベレリンの存在

Received July 1, 1959

Four gibberellins, A_1, A_2, A_3, and A_4, which are produced by a rice disease fungus, *Gibberella fujikuroi*, increase the height of a wide variety of plant species. Recently, it has been shown that substances with a biological activity similar to that of gibberellin A occur also in the extracts from immature seeds of various kinds of higher plants[1),2),3),4)]. One of these has been identified as gibberellin A_1[5)]. With regard to the mature dry seed Radley[2)] has reported the occurrence of such substances in wheat, French bean, and pea. No other direct evidence for the presence of gibberellins in mature dry seeds has been presented until now. The present paper describes the results of experiments designed to investigate whether gibberellins occur generally in mature dry seeds.

Materials and Methods

The seeds, which were examined for the presence of gibberellins, are given in Table 1.

Extraction: For extraction 50-100 gm. of dry seeds, which were previously ground in a mill, were covered with 500 ml. of petroleum ether and allowed to stand overnight at room temperature. The solvent was filtered off and the solid material was extracted at room temperature with two changes of 70% acetone for a total period of 48 hours. The combined acetone extracts were evaporated under reduced pressure. The resulting aqueous residue was acidified to pH 2.0 with phosphoric acid and extracted three times with 100 ml. portions of ethyl acetate. The ethyl acetate solution was then extracted three times with 100 ml. portions of 1 M phosphate buffer of pH 7.0. This phosphate buffer solution was acidified to pH 2.0 with phosphoric acid and extracted again three times with ethyl acetate. The ethyl acetate extract was dried by anhydrous sodium sulfate overnight and the solvent was distilled off under reduced pressure. The resulting residue was taken up in a small volume of acetone for paper chromatographic studies. Both weight and number of seeds, to which the amount of each extract applied to paper sheets was equivalent, are given in brackets with the name of species in the legend of Fig. 2.

The methods of paper chromatography and bioassay are essentially similar to those used in the previous paper[4)].

Paper chromatography: Ascending chromatography on Tôyô No. 50 filter paper

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Table 1. Species surveyed.

Family	Species
Compositae	*Helianthus annuus* (sunflower)
	Lactuca sativa (head lettuce)
Cucurbitaceae	*Cucumis sativus* (cucumber)
	Citrullus vulgaris (water melon)
	Luffa cylindrica (towel gourd)
Solanaceae	*Lycopersicon esculentum* (tomato)
Convolvulaceae	*Cuscuta japonica* (dodder)
	Pharbitis Nil (morning-glory)
	Quamoclit pennata (cypress vine)
	Ipomoea Batatas (sweet potato)
	Calonyction aculeatum (common moonflower)
Sapindaceae	*Cardiospermum Halicacabum* (balloon vine)
Rutaceae	*Citrus Natsudaidai* (natsudaidai)
Tropaeolaceae	*Tropaeolum majus* (garden nasturitium)
Leguminosae	*Lupinus luteus* (yellow lupine)
	Vicia Faba (broad bean)
Rosaceae	*Malus pumila* (apple)
Cruciferae	*Raphanus sativus* (Japanese radish)

was carried out with the solvent mixture of *iso*-propanol/water/ammonia (10 : 1 : 1) at about 25° until the solvent front was 28 cm. from the starting-line. The paper sheet was dried and then cut transversely into 14 equal strips. Each strip was again cut into small segments and placed in beakers 3 cm. in diameter and 7 cm. in height for determining its biological activity.

Bioassay: The activity was measured by the rice seedling method. The technique has been described in detail in the previous paper[4]. Briefly, five rice seedlings (var. Aichi-Asahi), whose coleoptiles attained about 1 mm., were planted in each beaker and allowed to grow under the ordinary daylight conditions at 25-28°. They were supplied with 0.5 ml. water every day. The length of the second leaf sheath was measured after 7 days. A result typical of the rice seedling method is given in Fig. 1. The lower limit of sensitivity was at 0.05 mg./l. gibberellin A_3.

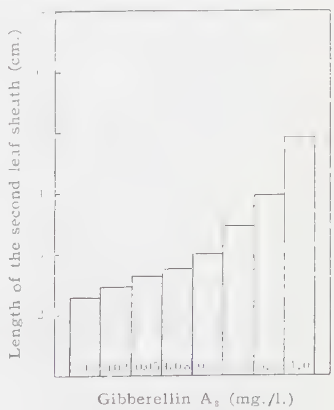

Fig. 1. Response of the second leaf sheath of rice seedling to gibberellin A_3.

Results and Discussion

The results of experiments, which are carried out to show the distribution of gibberellins in various mature dry seeds, are summarized in Fig. 2 as histograms

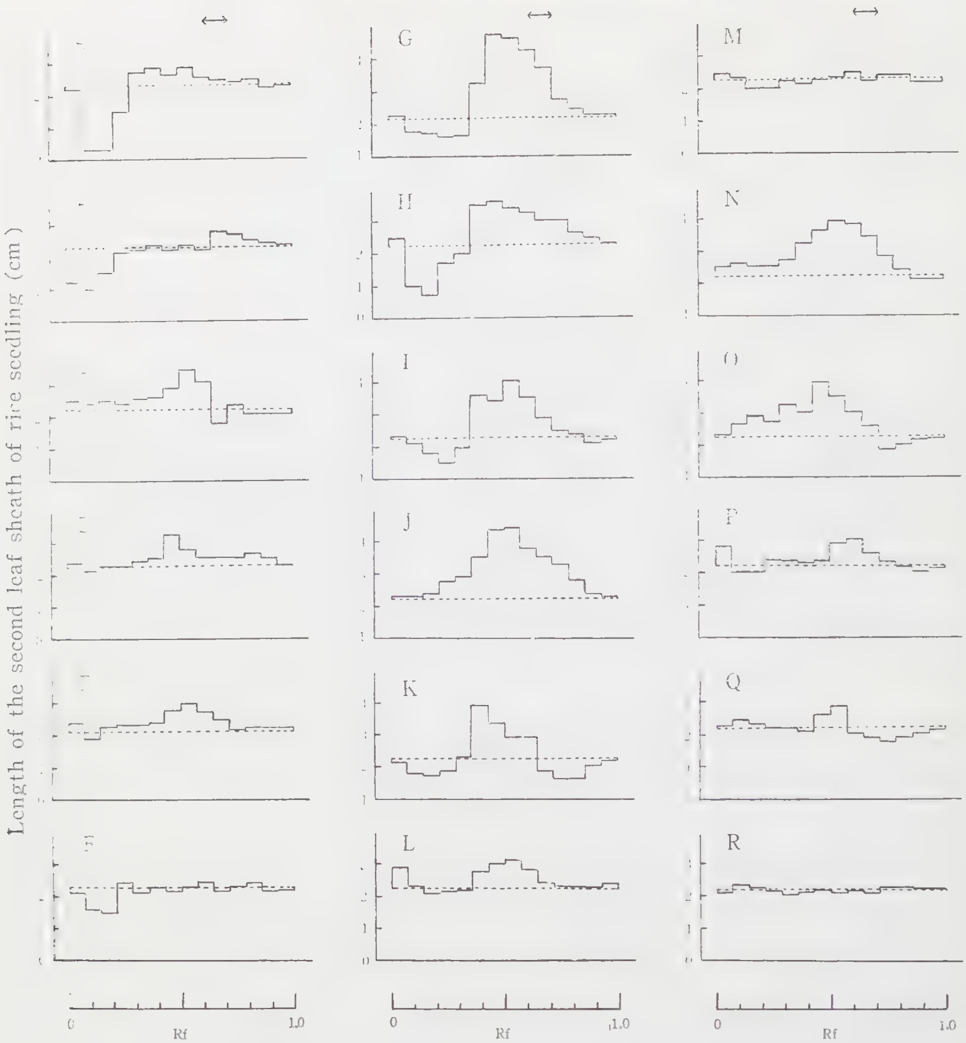

Fig. 2. Histograms showing gibberellin activity of extracts from mature dry seeds after paper chromatographic development with ammoniacal *iso*-propanol. Broken lines denote water controls. Arrows at the top of the histograms indicate the position of gibberellin A.

A, *Helianthus annuus* (34 g./700 husked seeds); B, *Lactuca sativa* (30 g./36,000 seeds); C, *Cucumis sativus* (70 g./3,600 seeds); D, *Citrullus vulgaris* (45 g./1,100 seeds); E, *Luffa cylindrica* (110 g./1,150 seeds); F, *Lycopersicon esculentum* (55 g./18,000 seeds); G, *Cuscuta japonica* (7.5 g./1,200 seeds); H, *Pharbitis Nil* (3.5 g./60 seeds); I, *Quamoclit pennata* (5.5 g./350 seeds); J, *Ipomoea Batatas* (3 g./160 seeds); K, *Calonyction aculeatum* (12.5 g./70 seeds); L, *Cardiospermum Halicacabum* (60 g./730 seeds); M, *Citrus Natsudaidai* (45 g./370 seeds); N, *Tropaeolum majus* (28 g./300 seeds); O, *Lupinus luteus* (10 g./110 seeds); P, *Vicia Faba* (30 g./15 seeds); Q, *Malus pumila* (57 g./1,140 seeds); R, *Raphanus sativus* (100 g./6,500 seeds).

of the length of rice leaf sheath. The horizontal broken lines in the figure represent the growth of controls so that leaf sheath elongations greater than this represent growth promotion and those below represent inhibition.

On every chromatogram of the extracts, except for that of *Lycopersicon esculentum* (Fig. 2-F), *Citrus Natsudaidai* (Fig. 2-M) and *Raphanus sativus* (Fig. 2-R), the growth-promoting activity was detected. Although the active substances were caused variations in both the Rf value and the tailing on the chromatograms because of the presence of large quantities of impurity, the growth-promoting zone lay near the expected position of gibberellin A. As described in previous papers[4),6)], the rice seedling method is specific to gibberellins, and indole compounds are inactive in this bioassay. Therefore, the growth promotion in this zone is considered to be due to the presence of gibberellins.

On the chromatograms of *Cardiospermum Halicacabum* (Fig. 2-L) and *Vicia Faba* (Fig. 2-P), another weak growth-promoting activity was shown near the starting line. A similar active zone was also found with some extracts of immature seeds of leguminous plants[4)].

No promoting activity could be detected on the chromatograms of the extracts of *Lycopersicon esculentum*, *Citrus Natsudaidai* and *Raphanus sativus*. This does not necessarily imply the absence of gibberellins in these seeds, but may be due to very low concentrations of such substances on the chromatograms.

The approximate concentrations of gibberellins present in various seeds, which were determined by comparison with a known quantity of gibberellin A, are listed in Table 2. These values may be lower estimates than the amount of promoters actually present in the extracts, since the contaminating inhibitors in the samples had a strong effect on the growth of the test plant.

It should be noted that there were remarkably higher concentrations of gibberellins in the seeds of climbing plants. The seeds of members of the Convolvulaceae, such as *Cuscuta japonica*, *Pharbitis Nil*, *Quamoclit pennata*, *Ipomoea Batatas* and *Calonyction aculeatum*, contained the substance with activity equivalent to 10–100μg. gibberellin A per 100 g., and that of *Tropaeolum majus* and *Cardiospermum Halicacabum* gave values equivalent to 1–10μg. gibberellin A per 100 g. dry seeds. Gibberellins in members of the Cucurbitaceae were a con-

Table 2. Gibberellin concentrations found in mature dry seeds.

Species	Approx. conc. (μg. gibberellin A equivalents/100 g.)
*Helianthus annuus**	0.5
Lactuca sativa	0.25
Cucumis sativus	0.85
Citrullus vulgaris	0.4
Luffa cylindrica	0.3
Lycopersicon esculentum	?
Cuscuta japonica	47
Pharbitis Nil	30
Quamoclit pennata	22
Ipomoea Batatas	90
Calonyction aculeatum	10
Cardiospermum Halicacabum	1.5
Citrus Natsudaidai	?
Tropaeolum majus	7
Lupinus luteus	11
Vicia Faba	2
Malus pumila	0.3
Raphanus sativus	?

* Husked seeds were used in the case of *Helianthus annuus*.

centration of the order equivalent to 0.5-1μg. gibberellin A in 100 g. seeds. *Lupinus luteus* and *Vicia Faba* have no climbing habit under ordinary conditions. However, they are members of the Leguminosae which includes a number of climbing plants. In fact, the seeds of above two species were comparable to that of a climbing plant, *Tropaeolum majus*, in content of gibberellins. Radley[2] has reported that there is no evidence of a difference between dwarf and tall varieties of pea in content of gibberellins. The results of present investigations, however, indicate that a much higher concentration of gibberellins occurred in seeds of the families including climbing plants.

Summary

The occurrence of gibberellins in the extracts of mature dry seeds from 18 different species of dicotyledonous plants was examined by means of paper chromatography and rice seedling method.

Gibberellins were evidently found in the seeds of sunflower, head lettuce, cucumber, water melon, towel gourd, dodder, morning-glory, cypress vine, sweet potato, common moonflower, balloon vine, garden nasturitium, yellower lupine, broad bean, and apple, but not in those of tomato, natsudaidai, and Japanese radish.

The seeds of the Convolvulaceae contained considerably higher concentrations of gibberellins and their contents were equivalent to 0.1-1μg. gibberellin A per gram of dry seed.

The writer wishes to thank Prof. T. Miwa and Dr. T. Hayashi for their guidance.

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摘 要

先に，著者は，ペーパークロマトグラフィーに，イネ苗試験法を併用して，マメ科の未熟種子にジベレリンが含まれていることを明らかにした。本報告には，同様な方法を用いて，双子葉植物の完熟種子にも広く，ジベレリンが含くまれているか否かを調査した結果が記されてある。

ヒマワリ，レタス，キウリ，スイカ，ヘチマ，ネナシカズラ，アサガオ，ルコウソウ，サツマイモ，ヨルガオ，フウセンカズラ，ノウゼンハレン，ルーピン，ソラマメ，リンゴの完熟種子にはジベレリンが含まれていた。トマト，ナツダイダイ，ダイコンの種子では検出しえなかつた。一般に，「よじのぼり植物」の種子には，ジベレリンの含量が多く，特に，ヒルガオ科の完熟種子は，1 g. 当り，0.1～1μg. のジベレリン A に相当する量があつた。

Responses of *Pharbitis Nil* Chois. to Gibberellin with Special Reference to Anatomical Features

by Mitsuro OKUDA*

[illegible]

Received July 7, 1959

Gibberellin induces stem elongation, which is more remarkable in dwarf strains than in tall ones generally. The induced stem elongation is said by some investigators[1),2),3),4)] to be attributable mainly to cell elongation in the internode of the plant. On the other hand, it is reported that the elongation is due to cell division in the internodal cells[5),6)]. According to other authors not only the length of cells but also the number of cells is increased by the treatment[7)].

In the present paper some observations on the seedlings of *Pharbitis* treated with gibberellin with special reference to the structure of stem will be reported.

Materials and Methods

The materials used were two strains of Japanese morning glory, *Pharbitis Nil* Chois. One was "Kidachi", a dwarf strain, and the other was "Violet", a tall one. The seeds were treated with conc. H_2SO_4 for 45 minutes and washed in running water for 24 hours. They were sown in wooden boxes, and kept under continuous illumination supplemented with incandescent filament lamps at night. 0.01 cc. of an aqueous solution of gibberellin** was given two times, that is, after two and five days from the sowing of the seeds. One drop of the solution was applied to the growing point by the use of a micropipette. Control plants were given one drop of distilled water. The plants were harvested ten days after the last treatment and observations were made.

Results

a) Responses of various organs: Soon after the application of gibberellin solution—after some six hours or so—the effect on elongation of hypocotyl, epicotyl and cotyledonary petiole is observable, and it is strengthened with the lapse of time. The cotyledon treated with gibberellin also expands remarkably and becomes pale in color. The response increases with increasing concentrations of the solution

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** Gibberellin used in these experiments was supplied by Japanese Gibberellin Research Association and mainly consists of gibberellin A_3.

used. Among the organs the epicotyl reacts most sensitively. As shown in Fig. 1, two strains differ in their reactivity. Ten days after the last application of gibberellin the control plants of the two strains differ in height strongly, whereas the treated plants do not, especially in the plants treated with high concentration of gibberellin. The epicotyl of dwarf strain treated with 1000 p.p.m. gibberellin solution became nine times as long as that of the control plants, but in tall strain, it attained only to 2.4 times.

b) Rate of differentiation of leaf primordia: Further question arises whether the differentiation rate of leaf primordia at the terminal bud is accelerated by the application of gibberellin. To decide this point, two-day-old seedlings were given 100 p.p.m. gibberellin solution, and 0, 2, 4 and 8 days after the treatment, the leaves initiated on the terminal bud were counted under a binocular microscope.

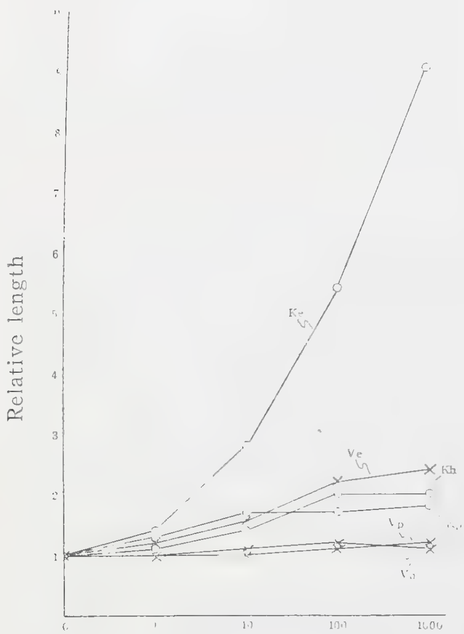

Fig. 1. Relative length of *Pharbitis* seedling treated with gibberellin as compared with the control. Sown on 12 July, treated on 14 and 16 July, and measured on 21 July. Length of the control plant of "Kidachi", hypocotyl: 46.1 mm., epicotyl: 19.8 mm., and cotyledonary petiole: 40.5 mm. Length of the control plant of "Violet", hypocotyl: 111.5 mm., epicotyl: 102.0 mm., and cotyledonary petiole: 50.8 mm. Kh, Ke, and Kp are hypocotyl, epicotyl, and cotyledonary petiole of "Kidachi" respectively. Vh, Ve, and Vp are the same of "Violet".

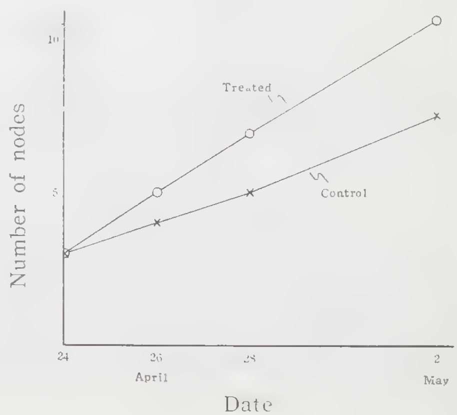

Fig. 2. Velocity of the differentiation of leaf primordia of *Pharbitis* seedling treated with gibberellin as compared with those of the control plants. Sown on 22 April and treated on 24 April.

At the beginning of the treatment three leaf primordia were observed in all individuals. After two days the treated and the control plants initiated five and four leaf primordia respectively. The difference between the number of leaf primordia initiated on the treated and on the control plants increased with time. After eight days the treated plants initiated 10.6 and the control plants 7.5 leaf primordia respectively. The results are shown in Fig. 2.

c) Anatomical observations: Hypocotyl, the first, the second, and the third internodes were observed anatomically. Ten days after the last treatment, both of the treated and the control plants were fixed in 70 per cent alcohol and hand-sectioned for the microscopic observation. The longitudinal, radial, and tangential dimensions of 30 cells from the cortical and pith parenchyma were measured with an ocular micrometer in treated and control plants of both strains.

The data secured are summarized in Table 1a. As the third internode was yet on the way of growing, the measurements were excluded from the table. In "Kidachi", the mean length of the shoot above the cotyledonary node was increased by 8 times by the application of gibberellin solution, and that of the hypocotyl was 1.3 times as compared with the control. The cell lengths of all organs of the

Table 1a. Length and dimension of cells from hypocotyl, the first and the second internode of *Pharbitis* seedlings treated with 1000 p.p.m. gibberellin as compared with those of the control.

Strain			Kidachi			Violet		
			Treated	Control	Treated as % of control	Treated	Control	Treated as % of control
Total length of epicotyl in mm.			228	26	887	197	44	448
Length × Diameter of hypocotyl in mm.			74×3.0	56×2.4	133×125.0	120×2.6	109×2.3	111×113.1
Length × Diameter of the 1st internode in mm.			82×1.5	14×2.3	586× 65.3	80×1.6	33×2.2	246× 72.7
Length × Diameter of the 2nd internode in mm.			67×1.3	7×2.0	957× 65.0	62×1.5	9×2.1	689× 71.4
Cell dimension in μ	Hypocotyl	cortex longitudinal	269	131	205	370	279	133
		cortex radial	49	52	95	50	41	122
		cortex tangential	41	53	78	51	45	113
		pith longitudinal	261	148	176	278	218	128
		pith radial	73	74	99	83	66	126
		pith tangential	75	73	104	70	67	104
	1st internode	cortex longitudinal	163	136	119	153	149	103
		cortex radial	18	50	36	15	30	50
		cortex tangential	26	43	61	22	32	69
		pith longitudinal	146	90	162	103	92	112
		pith radial	57	88	65	55	84	65
		pith tangential	54	69	78	51	73	70
	2nd internode	cortex longitudinal	105	108	97	85	76	112
		cortex radial	15	43	35	18	25	72
		cortex tangential	22	37	59	20	22	91
		pith longitudinal	96	74	130	83	72	115
		pith radial	41	69	60	38	49	78
		pith tangential	39	63	62	36	45	80

treated plants were longer than those of the control plants with the exception of cortex cell of the second internode. Radial and tangential diameters of the cells were decreased in almost all parts by the treatment. In "Violet", the same was the tendency, but the elongation of the stem and the cell was not so conspicuous as in "Kidachi". Radial and tangential diameters of the cells were decreased in all parts with the exception of hypocotyl, the cells of which had larger diameters.

The number of cells calculated from the mean length of organs and their cells is shown in Table 1b. The cells of the internodes increased in number enormously, especially in "Kidachi". The increase was more conspicuous in cortex than in pith, and also in the second internode than in the first. In the second internode of "Kidachi" the increase in the number of cells was found to be 9.85 times as large as that of the control plants in cortex and 7.38 times in pith, and in "Violet" 6.16 and 5.98 times were found respectively. The facts indicate that the internodal cell of the treated plant had repeated in the average 3 further cell divisions as compared with that of the control plant. The numbers of hypocotyl cells of both strains were reduced to a remarkable extent.

Table 1b. Relative number of cells in the internodes of *Pharbitis* seedlings treated with gibberellin as compared with those of the control plants.

Strain	Part of plant		Caculated number of cells in longitudinal direction		Relative cell number		
			treated	control	treated	:	control
Kidachi	Hypocotyl	Cortex	275	427	0.64	:	1
		Pith	284	378	0.75	:	1
	1st internode	Cortex	503	103	4.89	:	1
		Pith	560	156	3.59	:	1
	2nd internode	Cortex	638	65	9.85	:	1
		Pith	698	95	7.38	:	1
Violet	Hypocotyl	Cortex	324	391	0.83	:	1
		Pith	432	500	0.86	:	1
	1st internode	Cortex	523	228	2.29	:	1
		Pith	777	359	2.16	:	1
	2nd internode	Cortex	729	118	6.16	:	1
		Pith	747	125	5.98	:	1

The noticeable anatomical changes in addition to those in the size and in the number of the cells were also observed with supplying gibberellin solution. As shown in Fig. 3, only a few active meristematic cells are present in the cambial region of the treated plants. The cell walls of pericycle are thickened so conspicuously and lignified. The differentiation of the secondary xylem consisting of small and thick-walled elements is remarkably accelerated and so that a cylinder of lignified elements is formed around the stem.

In contrast to these characteristics in the treated plants there is an active cambial zone which is composed of thin-walled elements, and no wall-thickening is observed in pericycle in the control one. The differentiation of the secondary xylem

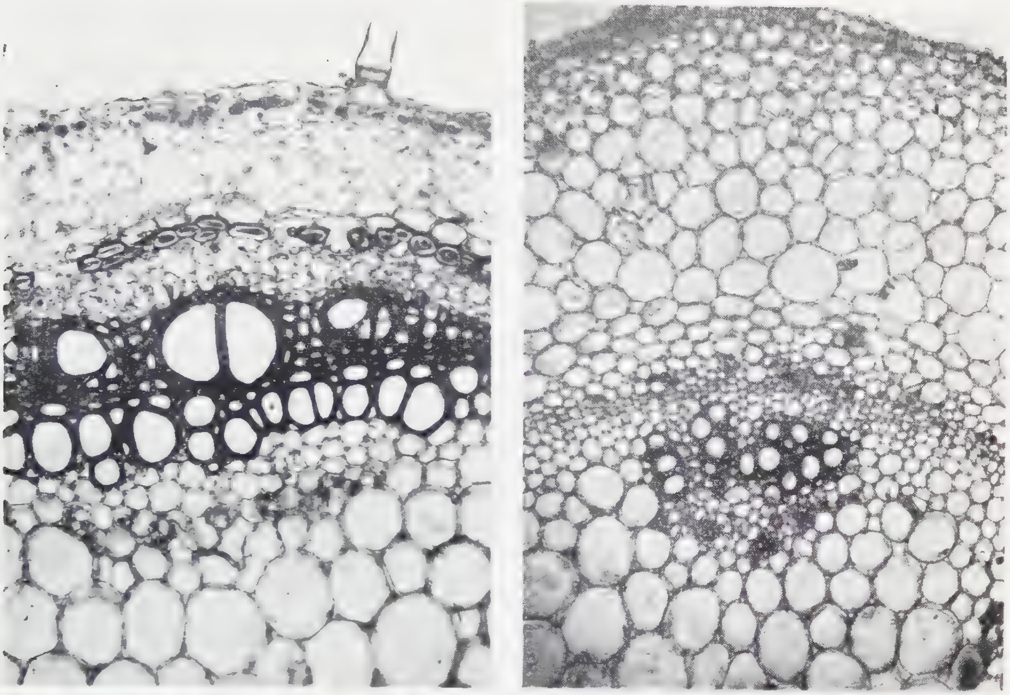

Fig. 3. Cross section of the first internode of *Pharbitis* seedling after ten days from the treatment with 1000 p.p.m. gibberellin solution. Left: Treated with gibberellin. Note the thickening of the cell walls in pericycle and xylem. 150×. Right: Non-treated control. 100×.

is not yet active and vascular bundles are separated by the thin-walled parenchymatous tissue.

Discussion

Gibberellin induced the remarkable stem elongation of Japanese morning glory, *Pharbitis Nil* Chois. and the effects were more remarkable in dwarf strain than in tall one. The elongation of all cells in longitudinal direction was induced by the treatment with gibberellin, but the radial and the tangential diameters were decreased in the internodes. Some transverse diameters of the cells in hypocotyl were, however, increased.

From the foregoing observations it is deduced that both cell division and cell elongation are affected by the treatment with gibberellin. In hypocotyl, in which the cell division had been almost completed at the start of the experiment, the residual cell division is arrested and its elongation is entirely due to the elongation of its constituents. In the shoot axis above the cotyledonary node, however, both cell division and cell elongation are concerned and its enormous elongation is attributable mainly to the division to the internodal cells. It seems thus likely that the reaction of the different organs to gibberellin varies in compliance with the age of the constituting cells.

The treatment with gibberellin brings about the changes of anatomical features, such as the acceleration of leaf differentiation at the growing point, the consumption of cambial activity, wall-thickening of the cells of pericycle and xylem. It is deduced from these anatomical changes in the stem of the treated plant that gibberellin brings about the early maturity of the plant body in spite of being in its early stage of the growing. Wilton and Roberts[8)] reported on the anatomical differences of the flowering and non-flowering stem of *Chrysanthemum* illustrating the structures as follows: "......There appear to be no meristematic cells present in the cambial zone. All the cells of the pericycle and phloem, except a few of the sieve tubes and companion cells, have come thick-walled and are stained by basic dye, crystal violet. The last formed secondary xylem consists entirely of small, thick-walled, usually oblong elements which appeared to be one type when seen in transverse section. ......The xylem and perimedullary zone are also stained by crystal violet". Similar anatomical features were also observed in the stem of *Pharbitis* plants treated with gibberellin, although there was a difference between non-flowering *Chrysanthemum* and non-treated *Pharbitis*, i.e. in the former plants there were thick-walled cells of the pericycle and pericyclic fibres but not in the latter.

In this connection it is a very interesting fact that gibberellin is able to induce the flowering of many plants having different photoperiodic behaviours, under critical or non-inductive photoperiodic condition. *Pharbitis Nil*, the material of the present study, is also reported to be promoted to flower by application of gibberellin on the growing point.[9)]

Summary

1) Dwarf and tall strains of Japanese morning glory, *Pharbitis Nil* Chois., were treated with gibberellin.

2) Differentiation of leaf primordia at the growing point is accelerated by the treatment.

3) The longitudinal diameter of the cells of the treated plants increases than that of the control, and both of radial and tangential diameters of the internodal cells decrease. Gibberellin affected on both cell division and cell elongation and the effects vary in compliance with the age of the treated organs.

4) Cambial activity is ceased almost entirely in the first internode of the treated plant, and the thickening of the cell walls of pericycle and xylem is remarkable. These thick-walled elements are lignified.

Acknowledgment

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摘　　要

アサガオつわい性種“木立”とつる性種“紫”の両種の芽生の生長点に 1000 ppm. のジベレリン水溶液を滴下し，処理後1[illegible]日目に観察した。

アサガオの茎の伸長に対するジベレリンの効果は，つる性種におけるよりもわい性種においていじるしい。細胞の大きさに対する影響は，[illegible]によって異なり，細胞の大きさは一般に縦の方向には長くなり，横の方向には小さくなる。ただし胚軸の細胞では横径の増加が見られる。

茎の伸長は，胚軸では主として細胞の伸長によるが，節間細胞では細胞の分裂と伸長との両方による。生長点における細胞の分裂，分化もジベレリン処理によって促進される。

ジベレリン処理をした植物の第一節間では，形成層の作用は活潑でなくなり，木部の細胞は細胞膜がいちじるしく肥厚している。この厚膜化した組織は茎の周辺に環状を呈している。細胞膜の肥厚の促進は内しょうの細胞にも見られる。

ジベレリン処理によって[illegible]，[illegible]膜の肥厚の顕著な[illegible]，ジベレリンの開花[illegible]かもしれない。

Dry Matter Production by *Chamaecyparis pisifera* in Winter

by Nobuo NOMOTO*, Hiromi KASANAGA** and Masami MONSI**

野本宣夫*・笠永博美**・門司正三**：サワラの冬における物質生産

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For the ecological purpose, it is desirable to know, how much dry matter production would be done by leaves of evergreen trees during the winter, and in this view their photosynthetic activity should be measured in that season. Much of the available information concerned with this problem is seen in Zeller's paper[1]. After Ålvik's method of colorimetric CO_2-measurement, some workers[2),3),4)] have investigated the subject whether net photosynthesis of evergreen plants is positive or negative in winter, with particular reference to the effects of frost. On the other hand, approaches were also made by studying the seasonal course of photosynthetic activity and seasonal pattern of dry matter production (Kusumoto[5),6)], Saeki and Notomo[7)], Bourdeau[8)]), especially detailed studies in *Picea excelsa* and *Pinus cembra* were carried out by Pisek and Winkler[9)].

In the present paper we will elucidate the features of CO_2-assimilation of twigs of *Chamaecyparis pisifera* (Sawara cypress), of which natural distribution is mainly restricted within the temperate region of Honshu, Japan, measured in the winter of 1954, and discuss the dry matter production in that period comparing them with those in spring and summer.

Material and Method

Sample twigs of *Ch. pisifera* were taken for each measurement from a hedge, grown from cuttings and about two meters high, at a suburban residential quarter of Tokyo. For this study, two kinds of branches were sampled from a tree, one had changed its leaf color from green to brown and the other kept its original green color throughout the winter.

For measurements of photosynthetic and respiratory rates, a modified Boysen Jensen's method[9)] was employed, and the same procedure with Saeki and Nomoto's[7)] was followed for excising sample twigs. The illumination was furnished by a 300 or 500 W reflector lamp with sky light and the flowing air through the assimilation chamber was regulated at a constant temperature during the experiment. The obtained values were corrected on the basis of 0.03 volume percent CO_2. The net and gross photosynthetic rates and the compensation point of each twig were deter-

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mined by defining the light-photosynthesis curve with a series of measurements. The chlorophyll content of leaves, after conversion of extracted chlorophyll into pheophytin, was determined colorimetrically[10].

Photosynthetic activity during winter

As referred previously, some twigs of *Ch. pisifera* kept on a brown color through the cold winter, and others remained green as ever. The chlorophyll content of these colored and green leaves, measured in the winter of 1954, was summarized in Table 1. During the late winter, from the end of February to early March, the chlorophyll content of the colored leaves was about 0.8 mg. per g. fresh weight of leaves,—that is, a half of the content of the green leaves in the same period. The leaves which had been changed in color through the cold months, gradually recovered to green in the spring (cf. Hiramatu[12]), increasing the chlorophyll content again. On the other hand, as to the green leaves whose color has been unchangeable in appearance throughout the winter, it may be said that the hibernal retreatment of chlorophyll content is not so remarkable. New scaly leaves of *Ch. pisifera* begin to develop late in May and their chlorophyll content increases rapidly until it attains the value of matured one.

Table 1. Comparison between chlorophyll contents in colored and green leaves of *Chamaecyparis pisifera*, in mg./g. fresh leaves. (The astericks indicate sprouted leaves).

Colored leaves		Green leaves	
Feb. 24	0.89	Feb. 25	1.65
Feb. 26	0.70	Feb. 26	1.62
Mar. 1	0.79	Mar. 3	1.45
Apr. 20	0.81	Mar. 5	1.99
Apr. 21	0.97	Apr. 12	1.38
Apr. 23	1.01	May 6	1.97
Apr. 26	1.04	May 7	2.11
Apr. 27	0.99	May 10	2.19
May 7	1.45	May 13	1.79
May 17	1.31 (1.08*)	May 15	1.89 (1.15*)

Fig. 1 shows the light-photosynthesis curves of those green and colored leaves obtained from late February to early March. The maximum net photosynthetic rate of colored twigs was 0.42 mg. CO_2/g. fresh weight/hr. at 10° on March 1, and this was only a half of 0.82 mg. of green ones on February 18 (at 10°). Another conspicuous difference between the colored and green leaves was the elevation of light compensation point in the

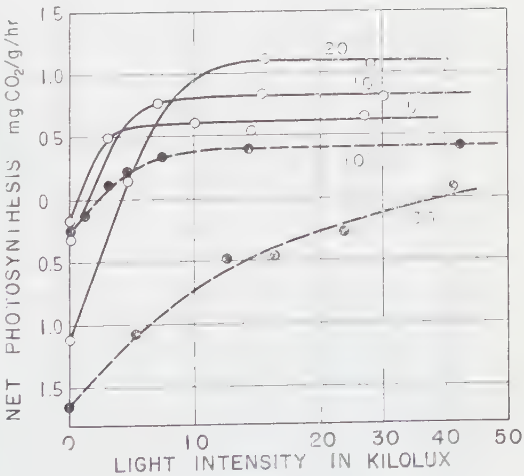

Fig. 1. Light-photosynthesis curves of *Chamaecyparis pisifera*. Solid lines show the curves of green twigs, obtained from Feb. 18 to Feb. 25, while broken lines those of colored ones, from March 1 to March 11.

former; that is, the compensation point of the colored leaves was ca. 2,300 lux at 10°, while that of the green ones, ca. 1,500 lux at the same temperature. The higher the temperature, the higher the compensation point, e.g. in the colored leaves at 20° ca. 8,000 lux of compensation point and at 30° even 40,000 lux were measured. Pavletić and Lieth[3] measured colorimetrically the light compensation point in winter in some conifers and evergreen species with a view to compare their frost sensibilities, and they confirmed that even at 3,200 lux, the highest illumination provided so far, there occurred no compensation in twigs of *Ch. nootkatensis* and some other conifers, suggesting that the compensation point may well be considerably high.

The productivity of the colored leaves in winter is characterized not only by their depressed maximum photosynthesis in saturated illumination, but also by extremely high light compensation point. The latter is partly due to the ineffective light absorption by the brownish pigments which exist in great quantity in chloroplasts of mesophyll, mainly palisade, cells in contrast with heavy chlorophyll decomposition and were confirmed as carotenoids by column-chromatography and spectrophotometry.—Red pigments in the autumnal leaves of conifers have been identified by Hida[13] as cyanidin and/or delphinidin, besides carotenoids. The above explanation was acertained by the fact that under the light transmitted through a red color filter (600-700 mμ)[14] the initial inclination of the photosynthetic curve of colored leaves coincided with that of green ones (see Fig. 2).

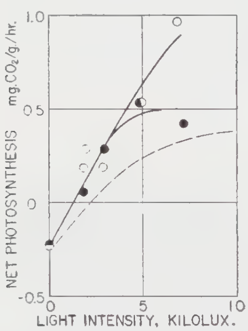

Fig. 2. Net photosynthetic rate of colored (solid circles) and green leaves (open circles) under the light transmitted through a red color filter (600-700 mμ); temperature 10°. Broken line indicates the same curve with that of the colored at the same temperature in Fig. 1.

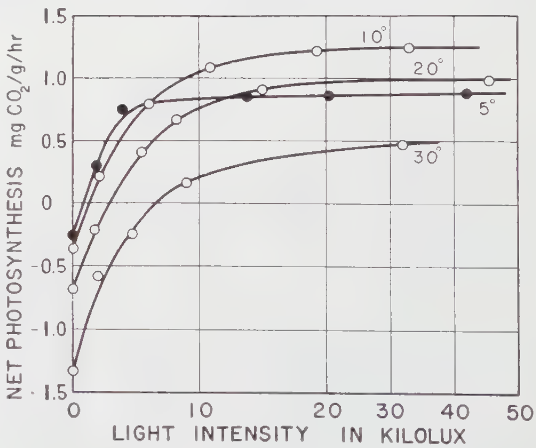

Fig. 3. Light-photosynthesis curves of twigs which had apparently recovered to green from colored condition, obtained from April 23 to April 30.

As the spring advances, the photosynthetic activity of colored leaves has clearly been restored as seen in *Picea* and *Pinus* by Pisek and Winkler[9], with the recovery of chlorophyll content. Curves shown in Fig. 3 were obtained for twigs

which already recovered apparently from colored condition in the last winter. The steeper slopes of curves in spring agreed with those of the green twigs in Fig. 1.

A comparison of dry matter production in winter with those in other seasons

The maximum net photosynthesis and respiration of twigs of *Ch. pisifera* determined in different seasons of the year were summarized in Table 2, excepting those of colored ones in winter. There is shown that the net photosynthetic rates measured at 20° and under saturated light condition increased from spring to summer, up to its maximum level in August, then markedly decreased in October. This

Table 2. Maximum net photosynthesis (a-r) and respiration (r) in mg. CO_2/g. fresh twigs/hr., and the ratio of a/r, of *Chamaecyparis pisifera*.

	Feb.			Apr.			May			Jul.			Aug.			Oct.		
	a-r	r	a/r	a-r	r	a/r	a-r	r	a/r	a-r	r	a/r	a-r	r	a/r	a-r	r	a/r
5°	0.7	0.2	4.5	0.9	0.2	5.5	—		—	—	—	—	—	—	—	—	—	—
10°	0.8	0.3	3.7	1.3	0.4	4.3	1.5	0.2	8.5	—	—	—	—	—	—	—	—	—
20°	1.1	1.2	1.9	1.0	0.7	2.4	1.6	0.5	4.2	2.3	0.8	3.9	2.7	0.5	6.4	1.2	0.4	4.0
30°	0.8	2.3	1.3	0.5	1.3	1.4	—	—	—	—	—		—	—	—	—	—	—

would give an impression that the duration of high activity in Sawara cypress is considerably shorter than those of Norway spruce and blue spruce examined by Bourdeau[6]. But, this trend is practically the same as that observed by Saeki and Nomoto in some deciduous and evergreen broad-leaf trees in Japan[7].

As mentioned above, the net photosynthetic rate in *Ch. pisifera* twigs was positive during the winter, even though it was at very low level, that is, about one third of those of the growing season. The net amount of dry matter produced by the photosynthetic system is determined by the balance of the income by photosynthesis over the expense by respiration in leaves in a given duration of time. Provided symbol p is the amount of organic matter produced by unit weight of leaves or twigs per day, it is, though approximately, shown by an equation

$$p=(a_t\,\tau-24\,r_t)\ C_6H_{10}O_5/6CO_2$$

where a_t and r_t are the gross photosynthetic and the respiratory rate of leaves or excised twigs at temperature $t°$ respectively, and τ is the photosynthetic hour. The daily production of *Ch. pisifera* twigs calculated based on the obtained results is shown in Table 3. For convenience of calculation, here the mean day length of three days, 1st, 11th and 21st, of respective months was applied to τ. From the Table 3 it is demonstrated that, in February the estimated productivity of green twigs is down below zero in higher temperature than 20°, in spite of its relatively high positive net photosynthetic rate, for the sake of the extremely high nocturnal respiration compared with the photosynthesis (see Table 2). It is of interest that the positive productivity of twigs during winter occurs only in the case of moder-

Table 3. The daily dry matter production, mg. $C_6H_{10}O_5$/g. fresh green twigs, in respective months.

	Feb.	Apr.	May	Jul.	Aug.	Oct.
Day length in hrs.	10.9	13.1	14.0	14.3	13.5	11.9
Mean monthly temperature °C	3.9	12.8	17.2	25.1	26.4	16.4
5°	3.1	5.5	—	—	—	—
10°	3.1	7.7	12.0	—	—	—
20°	−2.1	3.4	10.2	15.2	19.3	5.3
30°	−12.7	−5.3	—	—	—	—

ately lower temperature, and such low winter temperature is rather ordinary in Tokyo, as the mean monthly air temperatures of December, January and February are 5.7, 3.2 and 3.9°, respectively. Therefore, it is reasonable to assume that the daily production of green twigs in winter can reach upward of one sixth of that in summer, under the best conditions for dry matter production in respective sersons. On such an occasion in early March, the value of colored twigs fell to 1.3 mg. dry matter/g. fresh weight/day at 10°,—this is only one fifteenth of summer productivity.

The water content of sample twigs was about 55-60 per cent of fresh weight. Thus, it may be said that the green twigs can produce ca. 0.7 per cent dry matter of their own dry weight on a sunny and moderately cold day of winter, while the highest value on a summer day amounts to 4.5 per cent.

Summary

Photosynthetic activity and dry matter production during winter were investigated in the excised twigs of Sawara cypress *Chamaecyparis pisifera*, compared with those in the other seasons.

1. In winter, the chlorophyll content of colored leaves of this conifer, which were brown in appearance, had been reduced by half of that of the leaves remaining in green.

2. Net photosynthetic rate of green twigs in winter was positive, though it was at a low level of one third of summer rate. The colored twigs showed more remarkably depressed net photosynthetic rate, and their light compensation point ascended markedly.

3. A positive productivity of green twigs in winter can be observed only in the case of moderately low temperature; under an optimal condition in winter, it was estimated at 3.1 mg. dry matter/g. fresh weight/day, i.e. about one sixth of summer maximum productivity. The colored leaves could have a productivity of only one fifteenth of the summer value.

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摘　　要

サワラの切枝について冬期の光合成が[illegible]され，またその物質生産が春から夏にかけての値と比較されながら論ぜられた。冬にサワラは[illegible]に変色しているものと緑色のままのものとがあるが，前者の Chlorophyll 含量は後者の約 1/2 であつた。冬の見かけの光合成速度は緑色葉では 2 月中旬に実験温度 10°，光飽和のもとで 0.82 mg. CO_2/g.(生量)/hr. で 8 月の最大値の約 1/3 であつた。褐色葉では最大光合成速度がさらに[illegible]なつていることが[illegible]た。

[illegible]条件で得られた光・光合成曲線[illegible]かなり広い温度範囲で正の値を示しているにもかかわらず，正の物質生産の可能なのは 10° 附近の比較的低い温度条件下のみに限られていることがわかつた。冬の[illegible] mg. の物質生産を行なうことができるが，これは夏の最大値のほぼ 1/6 である。さらに褐色葉ではこの値は約 1/15 に低下している。

地中植物の地下部の深さの変動

——植物の生活型に関する二三の問題点，Ⅲ——

沼田　真*・浅野貞夫**

Makoto NUMATA* and Sadao ASANO**: Variation in the Depth of the Underground Part of Geophytes—Some Considerations concerning the Biological Types of Plants III.

1959年3月19日受付

1. はじめに

前報[1]において，われわれはまぎらわしい生活型のタイプについて論じたが，本報においては，とくに地中植物の地下部の深さの変動について検討した。Raunkiaer の生活型（休眠型）は，その一部は温度条件に対する反応型として，最高の休眠芽の位置により類型化したものであるが，多年生草本では地表植物(Ch)，半地中植物（H)，地中植物（G）の3者がとくに重要である。こうした生活型は一般に遺伝性の強いものであるが，一方環境によって変動する場合もある。環境条件によってGがHになり，あるいは深いGが浅いGになるというような事例があれば，これを環境指標として利用することも可能である。こうした見方にもとづいてGの地下部の深さの変動をしらべた。ものによってはたくさんの資料をうることが困難であったが，若干の事例について深さの変動に関する実態を明らかにしたので報告したい。

資料はおもに，千葉県安房郡清澄山，嶺岡山，鴨川町，および習志野の原野（1956～1958年）からとった。深さの変動は，変動係数と均質度係数[2]によってみることとした。

2. 測定例と問題点

A） 栄養繁殖型越年生草本

1. モミジガサ

従来Hとされた例[3]もあるが，完全なGで，繁殖型のうち根系型は R_{2-3}，地上茎は単一で冬期枯死する。樹陰，傾斜面に群生する。基質は一般に腐植にとむやわらかい壌土。清澄山における10月の状態はFig. 1a のようであるが，11月になると母本（親株）が枯れはじめ，地下部の基部から2～3本の棍棒状の地下茎がで，その先端はふくれて越冬芽をつける。芽は白色平滑，幅広い芽鱗でおおわれる。12月になると母本は完全に枯死し，Fig. 1b 左側でみるように根茎は途中からきれて離れ，冬芽を有する地下茎は母株から全く独立し，発達した根をもって翌年に備える。このよ

Fig. 1. Underground part of *Cacalia delphiniifolia*. The horizontal line shows the surface of the ground. a: A view in October at Mt. Kiyosumi. b: A view in December where the clonal connection breaks.

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Table 1. Position of the highest overwintering buds of four species

Plant	*Cacalia delphiniifolia*		*Ligularia tussilaginea*		*Euphorbia pekinensis* var. *japonensis*	*Vicia unijuga*	
Location	Mt. Kiyosumi (In case of Fig. 1a) 21, 10, 1956	Mt. Kiyosumi (In case of Fig. 1b) 24, 12, 1958	Mt. Kiyosumi (Under a forest canopy) 21, 12, 1958	Mt. Kiyosumi (On a sunny slope) 21, 12, 1958	Mt. Mineoka 7, 10, 1956	Mt. Kiyosumi (On a gentle slope) 12, 12, 1958	Mt. Mineoka (On a level ground) 7, 10, 1956
n	8	16	15	10	6	56	6
x	5.44	5.65	2.39	2.33	1.92	4.05	3.0
d	3.54	1.15	0.46	0.52	0.67	0.43	1.69
CV	0.804	0.384	0.347	0.309	0.334	0.393	0.537
CH	0.651	0.204	0.191	0.221	0.350	0.106	0.563

n: No. of individuals sampled, x: Average depth of the highest overwintering buds under the soil surface, d: 1/2 confidence interval, CV: Coefficient of variation, CH: Coefficient of homogeneity ($CH=d/x$)

から出た根茎の走る深さも，Fig. 1a のように必ずしも等しくないので，次の年に独立した個体の深さはまちまちである。もっともその程度を理論均質度係数の値[4] と比較してみると，Fig. 1b の場合などはほぼ均質とみてよいようである。変動係数も均質度係数とほぼ似た判断を与える。なお傾斜面では，土壌の流亡，落葉層の堆積などのため深さの変動をうけやすい。

2. サワギク（ボロギク）

牧野図鑑[5] には深山陰湿の地に生ずる越年生草本とあるが，これは一般の種子繁殖による越年生草本ではない。本種のほか，前記のモミジガサ，ヤブレガサなどの草本は，あるいは多年生あるいは越年生と記載されているが，じつはそれらの中間の性質をもっている。すなわち生活様式が，前記モミジガサなどと同様に晩秋に母本は地下部とも枯死し，母本に連続している2～3本の地下茎は，たとえば Fig. 2a の点線部よりも先の部分が切れて離れ，その先端の越冬芽が翌年伸長し，Fig. 2b のように独立した個体となるのである。このようにしてできる新個体が種子繁殖にもとづくものでない点が特色である。それがさらに，夏期に一方向に，白色で表面の平滑な軟かい地下茎を数本だし，同じように晩秋に何個体かにわかれる。冬期Fig. 2b のような形のものが，前年の母本のまわりに点在するようにして翌年には個体数が2～3倍になる。また母本するときあたかもふつうの越年生草本のようにみえるのである。

このサワギクもモミジガサ型の生活型の一例としてあげたのであるが，このような dead centre[6]をつくるタイプの栄養繁殖型越年生草本は R_3 型の一つの特色ある繁殖型と考えられる。

B) 地下部の位置の変動例

3. ツワブキ

樹陰，半陰地に多いが，時には岩上，日あたりの向陽地にも生ずる。一般に海浜に近い山野（とくに傾斜地）に多い。基質は腐植にとむ壌土が多く，大抵の場合附近の地表面や葉柄のもとに落葉が堆積する。越冬芽は葉柄の基部およびその年の地下茎につき，芽鱗は厚く，基部が広く互いにおおい，かつ表面には白色の綿毛がおおう。向陽地のものは，時に年内に越冬芽が伸長することがあるが（Fig. 3a)，一般には少い。最高の越冬芽の位置は樹陰のものも向陽地のものもあまり差異がない（Table 1）。

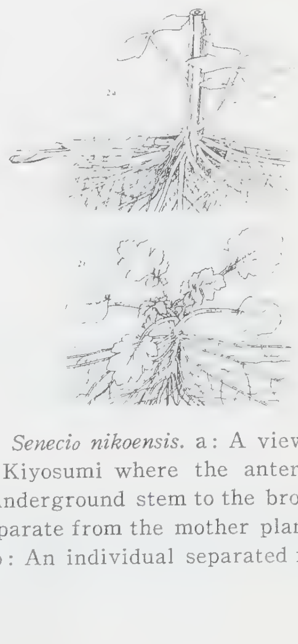

Fig. 2. *Senecio nikoensis*. a: A view in July at Mt. Kiyosumi where the anterior part of an underground stem to the broken line will separate from the mother plant afterward. b: An individual separated from the clone.

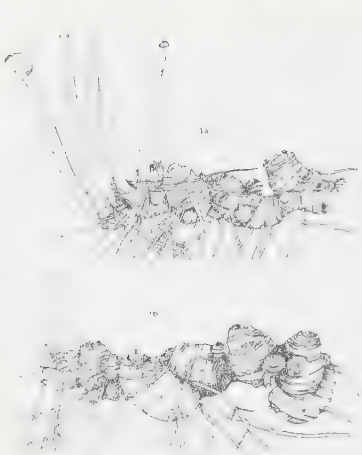

Fig. 3. *Ligularia tussilaginea.* a: A view where some overwintering buds elongated before the end of the year. b: A normal view in winter.

4.　タカトウダイ

半陰地，陽地の傾斜面をこのみ，埴壌土のようなところに多い。Fig.4aのように，地下部は肥大して不規則に分岐する肥大根であり，地上部は1本もしくは2～3本叢出する。越冬芽は，平滑な芽鱗におおわれ，地下部に不規則に散生する。時にFig.4bのように，族生した地上茎の下部に芽をつけるHの形式をとるが，この芽は活力がなく，十分生長せずにおわる。いずれにしても Table 1 [illegible]

Fig. 4. *Euphorbia pekinensis* var. *japonensis.* a: A normal view. b: An unusual type like the hemicryptophyte.

5.　[illegible]

[illegible]の陽地，半陰地に[illegible]，埴壌土[illegible]に多い。地下茎[illegible]地中深く入り，かつたくさん枝分れし，木質化し，越冬芽が不規則に着生している。白色ないし帯赤色の芽鱗は平滑で軟いが，時に地上部に露出するものは淡緑色を呈する。越冬芽は晩秋すでに伸長をはじめる。1958年12月の測定でも，G型のもの56に対し，すでに越冬芽の露出するもの5をかぞえた(芽の高さ平均 5.1cm.)。芽の深さは Table 1 のように，平均値では斜面の方が深いがこの資料では平坦地との間に有意差はみられない。Fig. 5a は典型的な型で根は不規則に分岐し，地中深く斜走する。冬芽は白色で長く伸び，地表に少しでることもある。Fig. 5b は粘土質の固い土壌のもので根が比較的浅く，かつ地上茎が地表の附近でさかんに分岐し，冬芽の位置は浅くかつ長く伸び，先端が地上に[illegible] H [illegible]ることもある。また時には Fig. 5b のように，昨年枯死した茎[illegible] Ch [illegible]Gと[illegible]

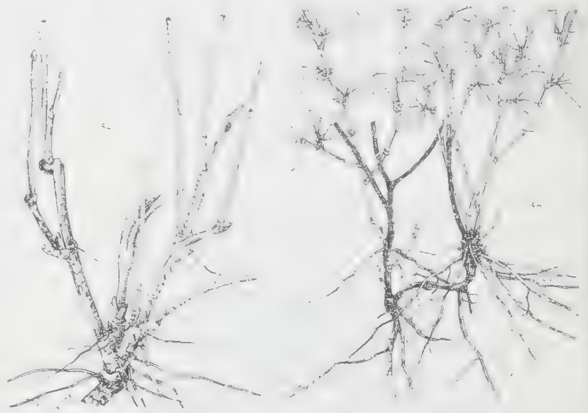

Fig. 5. *Vicia unijuga.* a: A normal view. b: An unusual type like the chamaephyte where the overwintering shoots above the ground wither in a short time.

6.　ヌスビトハギ

生育地は山地，原野，路傍，向陽地，樹陰などさまざまで，基質についても，壌土，砂壌土，腐植土などいろいろある。このように生育地への適応性が大きいためか，生活型も変動が大きく H か G かまぎらわしい場合がしばしばある。同属のマルバヌスビトハギも同様であるが，ヤブハギは明らかに H である。この種には往々にして Ch の形をとるものもあるが，茎の下部に着生する冬芽は来春一本立ちに完成しない。前報[1]のイノコズチと同様である。Fig. 6 のように，地下茎に小形の越冬芽を多数つける。Table 2

Table 2. Position of the highest overwintering bud of *Desmodium racemosum*, and percentages of geophyte and other types

Location	Mt. Kiyosumi (On a gentle slope) 22, 10, 1957	Mt. Kiyosumi (On a slope with rich litter) 22, 10, 1957	Mt. Kiyosumi (On a level ground) 22, 10, 1957	Mt. Mineoka (In a half shade) 7, 10, 1956
n	10	41	66	11
$\bar{x}$	1.32	0.57	1.56	1.41
d	0.38	0.34	0.28	0.50
CV	0.336	1.913	0.732	0.518
CH	0.282	0.603	0.180	0.357
$G\%$	90	87.58	99.92	100
$H\%$	10	12.20	0.03	0
$Ch\%$	0	0.22	0.05	0

Table 3. Position of the highest overwintering buds of four species

Plant	*Aster scabra*			*Sanguisorba officinalis*		*Adenophora triphylla* var. *tetraphylla*		*Gentiana scabra* var. *Buergerii*	
Location	Narashino (In a grassland) 29.12.1958	Narashino (At a sunny place) 29.12.1958	Mt. Mineoka (At an intermountainarea) 30.9.1956	Mt. Mineoka & Kamogawa (Onaslope) 30.9.1956	Narashino (On a level ground) 29.12.1958	Mt. Mineoka 30.9.1956	Narashino 29.12.1958	Mt. Kiyosumi (On a level ground) 21.12.1958	Mt. Kiyosumi (On a slope) 21.12.1958
n	11	6	8	29	31	15	6	6	35
$\bar{x}$	1.46	1.0	0.85	1.39	1.36	1.08	0.97	3.07	2.91
d	0.05	0.11	0.09	0.21	0.18	0.35	0.33	1.92	0.62
CV	0.048	0.100	0.013	0.403	0.382	0.592	0.330	0.597	0.595
CH	0.033	0.105	0.011	0.156	0.140	0.346	0.340	0.626	0.213

の下欄からみてわかろように，統計的にはGと考えられる

[illegible]

な[illegible]

とが CV や CH から推察される。

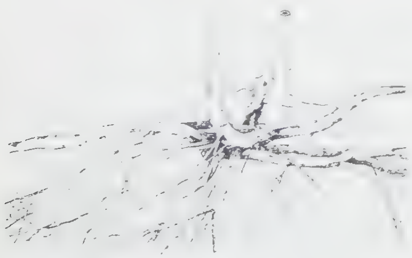

Fig. 6. *Desmodium racemosum*

7. シラヤマギク

晩秋に地上部は枯死するが，R_3 型の地下部には，表面平滑な越冬芽を多数着生する (Fig. 7)。壌土性の向陽ないし半陰地の平坦地に多く生ずる。芽が比較的大型で，また場所により浅いので (Table 3)，往々にして H と誤りやすい。

Fig. 7. *Aster scabra*

8. ワレモコウ

平坦地，傾斜地にいずれも生ずる。向陽地をこのみススキなどと共存する。地下茎は太く横走し，塊状根

をつける (Fig. 8)。 越冬芽は小形で，地下茎に多数生ずる。地下茎は地表面下浅いところを走るので H と誤認されやすく，傾斜地，平坦地など場所による差は少い (Table 3)。

Fig. 8. *Sanguisorba officinalis*

9. ツリガネニンジン

山野の壌土ないし多少埴壌土に近いところに多い。不規則な輪状の突起を有する円錐状の根があり，越冬芽は白色で表面平滑な芽鱗からなり，茎の基部すなわち円錐状の根の上端に数個生ずる。有機質の多いところでは浅根性になり，往々にして H と混同しやすい (Table 3)。

Fig. 9. *Adenophora triphylla* var. *tetraphylla*

10. リンドウ

平坦地には少く，緩および急斜面に多い。往々急傾斜の岩上で，土の堆積するところにも生ずる，傾斜地では地上茎も傾斜し，根は太くて四方にひろがる。越冬芽は円筒状で数個族生し，平滑な芽鱗をつける，向陽地の斜面では越冬芽が地表面に出て Ch 型になることがある。 平均値でみても多少浅くなる傾向がある (Table 3)。

Fig 10. *Gentiana scabra* var. *Buergerii*

3. 考察とむすび

従来，植物の記載において，生活型への注意はかなりおろそかにされてきたが，改めて吟味しなおしてみると，今までの数多くない観察も間違いをふくんでいることに気がつく。 G といっても，浅根性の場合 H とまぎらわしいものがある。地表面という境界はあっても，これを中心とした越冬芽の位置には明瞭な断絶はないので，当然こうした問題はおこる。[illegible]で最高の休眠芽の平均の深さがわずか1cm. でも2cm. でも，とにかく地中にあれば規定上 G としなければならない。“地中植物とは，地下部について休眠芽またはその先端の芽が地表面から離れて生ずるもの”[7] つまり芽の着生位置が地中にあることが重要な要件である。 地中植物でも *Lysimachia vulgaris* のように，地下部が地中深くあることはしばしばあるが そのような場合冬芽は横走根茎が地表すれすれまでにのびたその先端に，つまり深いところにある地下部ではなく，地表附近のものにつくことを Raunkiaer は注意している[8]。 Raunkiaer の休眠型はこうした “position characteristic”[8] によつて分類されている。つまり “Hは休眠芽が≪地表に≫位置する”と規定されているということは，≪芽の着生位置が地上に露出しない≫(別なところでは，≪まわりの土や，その植物自身の枯葉など (withered remains) によつて保護された地表に位置する≫ともかかれている[9]。) 程度ということであって，じっさいにはその範囲が問題になる 地表面を幾何学的な面と考えることは実際問題としては不可能なので0.5cm. 以内ぐらいの幅員は認めるべきであろうが，それにしても連続したものの境界であり，か

つ地表面は幾何学的な安定性をもっているものではないから，1個体について休眠芽の位置を厳密に計測するだけでは本質的な問題は解決しない。やはり統計的[illegible]

変動係数 (*CV*) や均質度係数 (*CH*) で変動の大いさをみようとしたが，これらはもちろん取りあつかっ[illegible]い。しかし，ヌスビトハギやフタバハギのようにかなり変動のあるものと，ツワブキのように変動の小さい[illegible]

についての判定では Ch や H となることがあつても，その土地の一般のタイプとしては統計的に G となるといつた事態があらわれる。dead centre をつ[illegible]いての生活型の判定を誤る場合がある。こうした点から生活型の判定には，ある特定時期に少数個体で判定[illegible]位置の指標価値は，ヌスビトハギのように変動の大きいものでは多少認められたが，一般に想像されるほど[illegible]

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Summary

In the description of plants some observations from the standpoint of the life-form were not uncertain. We discussed some of ambiguous types of life-forms in the previous paper (Report II, 1956). After Raunkiaer, "Geophytes include land plants whose surviving buds or shoot-apices are borne on subterranean shoots at a distance from the surface of the ground" (1934, p. 64). On the contrary, "all hemicryptophytes have their surviving buds or shoot-apices situated in the soil-surface" (p. 41), and "the buds do not come above the surface of the ground" (p. 40). The difference between the expressions "at a distance from the soil-surface" and "in the soil-surface" is sometimes very nice, especially in shallow geophytes.

We measured as many individuals of doubtful species as possible at different habitats (Figs. 1-10, Tables 1-3). That explains the matter there are variable species such as *Desmodium racemosum* and *Vicia unijuga*, and little-variable ones such as *Ligularia tussilaginea*. In the former a geophyte in a statistical meaning is sometimes like a hemicryptophyte or a chamaephyte as shown in Table 2. And the type of the life-form of species forming a dead centre (Figs. 1-2) is too apt to be misjudged in a season when the clonal connection breaks. The position characteristic of the surviving bud of variable species (Table 2) is considered to some extent as an indicator criterion, but in other plants the value as an indicator is little.

サンショウモの日長反応に関する研究

(II) 数種の有機酸の吸収が日長効果に及ぼす影響

柴　田　　治*

Osamu SHIBATA*: Studies on Photoperiodic Responses of *Salvinia natans* (II) The Influences of Organic Acids of Citric Acid Cycle on Photoperiodic Responses

1959年4月15日受付

植物の日長反応において，炭水化物がかなり重要な働きをしていることはすでに多くの人々[1]によって確められており，更にその働きが解糖系の前半部に依存しているのではないことも示された[2,3]。また，オナモミでは明反応がクエン酸回路内の有機酸によっておきかえられるのみでなく，その花芽形成も強められると報告されている[4]。

著者も炭水化物の代謝阻害が日長効果を著しく減少させる[3]ことから，日長反応におけるその代謝物質としての有機酸の重要性を考慮している。この推察を確めるため，日長処理下にクエン酸回路内の有機酸を植物に吸収させたところ，はなはだ興味ある結果がえられたのでそれを報告する。

材料および方法

実験材料および方法は前報[5]とほとんど同様であったが，以下に簡単に記す。

0.1% クノップ液で培養されていたサンショウモを材料とし，日長処理前後の培養はすべて電燈補光による長日下で行なった。

短日処理は3対の気葉が完全に開いた時から，8時間明期，16時間暗期で3回行なった。処理期間中に開く気葉はすべて展葉前に切除した。

ここで用いたのはクエン酸，コハク酸，フマール酸，リンゴ酸のクエン酸回路内の酸4種である。これらの暗期間の吸収が日長効果にいかなる影響を及ぼすかを調べるため，16時間の暗期だけ所定濃度に有機酸を溶かした培養液中に植物をおき，明期にはこれを水洗後これらの酸を含まぬ培養液に移した。

日長効果は反応個体比と平均胞子果数，またこの平均胞子果数から求めた阻害度と更に胞子果の成形の状態とによって比較し，その優劣を論じた。

対照とした植物は短日処理のみで，薬剤処理は全く行なわなかった区のものである。

結　　果

実験1　各酸を単独で吸収させた時の結果は第1図に示した。これらの結果から，ここで用いた4種の酸は全く異った効果をあらわす2組に分けられることがわかった。即ち，クエン酸は試みた範囲ではすべての濃度で日長反応を阻害し，濃度の減少につれて阻害度は徐々に減少した。これに反して，コハク酸，フマール酸，リンゴ酸はいづれも高濃度で阻害したが低濃度では促進し，10^{-5} または 10^{-6} M で日長効果は最大となった。阻害，促進いづれの場合にも同一濃度ではコハク酸の最大値はフマール酸リンゴ酸各々の2倍あるいはそれ以上の値を示し，コハク酸は他の2種の酸より日長反応の促進にいちじるしい影響を与えていた。

酸の種類またはその濃度は胞子果数の増減のみでなく，その成形の状態にも影響していた。クエン酸添加の場合にはすべての濃度で胞子果の成形は対照区のものより劣り，10^{-7} M で不完全ながら成形したものがみられたのみでこれ以外の濃度ではほとんど原基を分化した程度であった。他の酸では 10^{-3} M でほとんどの個体が原基のみであった以外は，他のすべての濃度で成形，生長いずれもが対照区のものより優っていた。すなわち，濃度の減少につれて完全成形のものが多くなり，またその生長も盛んでかなり大型のものが着果していた。ことにコハク酸の場合にはいちじるしかった。

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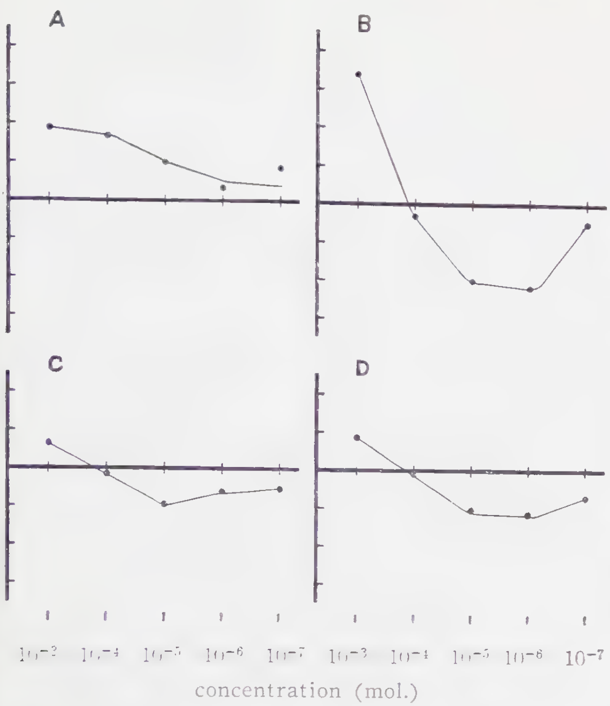

Fig. 1. Relations between the concentrations of acids of citric acid cycle and the photoinductive effects of *S. natans* : A ; citric acid, B ; succinic acid, C ; fumaric acid, D ; malic acid

実験２　実験１の結果から，クエン酸と他の酸とは日長効果に及ぼす影響をまったく異にするように考えられたので，この点をさらに確実にするために次の如き実験を行なった。

クエン酸濃度を10^{-4}Mとした培養液に，さらに各々10^{-4}M [illegible]

[illegible]の酸も添加した時にはそのような阻害はまったくあらわれなかった。コハク酸の同時添加の場合にはコハク酸のみの時とわずかに相違したのみであったが，フマール酸，或はリンゴ酸の同時添加ではこれらの酸が10^{-4}Mで各々単独の時よりかなり促進された。

胞子果の長生はクエン酸のみの場合と他の酸も同時に加えた時とで顕著な違いはなかった。

実験３　10^{-4}Mとして各々の酸を与えると同[illegible]して，つねにコハク酸が多量に存在することが考えられる。

[illegible]ン酸は単独では日長効果をいちじるしく増大さaせる[3]が，5×10^{-6}Mでは阻害度は－30％となったこれにクエン酸の同時添加はまった[illegible]れ，阻害度はいづれも－25％前後であった。胞子果の生長は有機酸も同時に吸収させた時よりマロン酸のみの場合の方が多少優っていた。

実験４　[illegible]水化物の代謝[illegible]べて，またはその主要部が働きつつある時に更に添加された有機酸の効果である。したがってこれらが厳密な意味で日長反応における有機酸の必要度を示しているとは考えられない。それ故，NaF 阻害による炭水化物の欠乏下で有機酸を与[illegible]

NaF を10^{-4}M として暗期のみ培養液に加えた時，[illegible]酸を加えても補償されたが，ここでもクエン酸と他の酸とは補償効果の大きさで異なっていた。すなわち，酸濃度を各々10^{-4}Mとした時の阻害度はクエン酸では24.1％，コハク酸，フマール酸，リンゴ酸で各々9.4％，13％，－３％となり，クエン酸以外の酸の方がその補償の程度はかなり大であった。ことにリンゴ[illegible]

Table 1. Effects of the certain acids combined with citric acid on the photoperiodic responses of *S. natans*.

Solution	Organic acids added to the culture solution*	Inhibition-grade (%)
1	Citric and succinic acids	－7
2	〃 and fumaric acids	－14
3	〃 and malic acids	－17

* The concentration of each chemical was adjusted to give 10^{-4}M.

果の生じた率はコハク酸で対照区のものより優っていた以外は，いずれも対照区程度かあるいはそれよりやや劣っていた。

考　　察

各々単独のコハク酸，フマール酸，リンゴ酸はいずれも適量で日長効果を強めたが，コハク酸の場合にはこの働きはことに大であった。炭水化物の欠乏下でこれらが与えられた時にリンゴ酸による効果がもっとも顕著であったことを除いては，他の場合にもほとんど同一傾向であった。コハク酸のこのような働きは，詳細には続報[3]で報告する予定であるがコハク酸脱水素酵素の阻害剤であるマロン酸が日長効果を強めることと全く同一の理由によるものと思われる。

生殖相の分化に際して，クエン酸回路内の有機酸がかなり重要な働きをしていることはすでに推察されているが，これらの結果からこの回路，または回路内の酸が日長反応において演ずる働きの重要さは一層確実なものとして示される。

ここで用いた酸はクエン酸以下すべて生体内では容易に，しかも相互に代謝されるはずである。そして通常はエネルギー代謝に必須の物質として用いられている。それにもかかわらず，日長効果に関してはクエン酸と他の酸の働きがまったく相反し，またクエン酸以外の酸が日長効果を強めた場合にしてもその働きはコハク酸でことにいちじるしかった。このような事実は通常は代謝に基本的なこれらの酸が，日長反応においては各々特異的な働きを有するようになることを示す。さらに，クエン酸阻害が他の酸の促進作用と拮抗することから次のような推論が可能であろう。すなわち，これらがすべて代謝に基本的な物質であることからして，クエン酸による阻害または他の酸によるその阻害の回復はクエン酸または他の酸が通常の酵素阻害剤の如く直接酵素活性を変じて生じたものではない。おそらく吸収によって常時と個々の酸の含量比が異なり，これが胞子果形成のための代謝過程に影響して間接的に生じたものであろう。したがって，クエン酸回路内の代謝平衡が破れることによって胞子果形成の阻害，または促進が生じたと考えられる。

日長反応に関して，与えた各酸の異なれる効果は酸を加えずに日長処理をした場合にも生体内で生殖相分化の一要因として働いている可能性がある。たとえば高等植物が暗所で固定した CO_2 はただちにクエン酸回路内のほとんどの有機酸成分としてあらわれるが，この固定による酸の増加の程度は個々の酸によって異なり，また暗期の経過時間につれても変動するという報告[6]が多数ある。それ故短日処理をうけた植物では，その含有する総酸量[7]とそれらの組成についても対照区のものとはかなり異なってくる筈である。ここでは培養液中から吸収された酸によって，酸処理区の方がこの対照区のものより当然特定の酸量は増加する。この場合吸収された酸が日長反応の過程で必要な物質と一致するか，あるいはその近縁物質であるならば，それによって日長効果が増大することもありうることであろう。しかし，このような酸量とその組成の変動が生殖相の分化にどの程度関係しているかは現在のところでは不明である。

この研究を行なうに当り，御指導と多大な御援助をいただいた中山包教授に深く感謝する

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Summary

1. The photoperiodic responses of a short-day plant, *Salvinia natans*, were studied by supplying with the organic acids of citric acid cycle.

The acids used were citric, succinic, fumaric and malic acids, added to the culture solution only during the dark period.

2. The developments of the sporocarps were inhibited by the citric acid of all

the concentrations tested, while in succinic, fumaric or malic acid of lower concentrations their developments were more or less accelerated, especially by the succinic acid.

3. The inhibiting effect of citric acid on the photoperiodic induction was not found when the other acids were also used at the same time.

These facts seem to suggest that the effect of citric acid on a process of the photoperiodic induction was antagonistic with that of the others, though all the acids used were available as the substitutes of carbohydrate required for the development of the sporocarps.

4. The malonic acid combined with the acids other than citric acid made some decreases in the photoinductive effect, whereas it was notably increased when the former acid was used alone. In citric acid, however, no such decreases were observed.

5. These facts seem to indicate that a relative quantity of the organic acids in the plants may be an important factor for an alternation from the vegetative to the reproductive phase.

放線菌胞子の電子顕微鏡による形態について

板　垣　史　郎*・古　川　　稔*・木　下　祝　郎*

Shiro ITAGAKI*, Minoru FURUKAWA* and Shukuo KINOSHITA*: The Morphological Observations with the Electron Microscope on the Spores of *Streptomyces*.

1959年8月9日受付

従来，放線菌 Genus *Streptomyces* の分類は，Bergey's manual[1] に記載されている Waksman-Henrici の方法にしたがっている，この分類によると有機培地上に生育させた場合にできる soluble pigment の色，aerial mycelium の色調などを主としており，形態的な特徴は比較的後の方の key として用いられているにすぎない。ところで，抗生物質生産という本菌種の特記すべき性質と，かなり変異をおこしやすい性質のため，新菌株，あるいは新菌種と称せられるものが続々発見報告され，分類上の混乱をきたしている。

Waksman らは前述のように色調を主体とした生理学的性質を重要な key としてあつかっているが，一方 Krassilnikov[2] は本菌種の分類にあたって，形態的形質を第1義とし，生理学的性質を第2義的に考え，その上で抗生物質の産生を特徴として分類を試みている。

また，最近 Pridham, Hesseltine および Benedict[3] は，Sporophore の形態によって7種に大別し，さらに色を取り入れた考へ方を発表している。

そのほか，分類に関する研究としては，Waksman,[4)5)6)7)8)] Lechavalier,[7)8)] Krassilnikov,[9)10)] Pridham および Gottlieb,[11)] Burkholder および Sun,[12)] Baldacci,[13)] Hesseltine ら[14)15)]Gause[16)] 岡見，酒井，[18)19)] 黒屋ら[20)21)] 秦ら[22 23 24)] 能美および長西，[25)26 27)] 能美，[28)] 早野，[29)] 山口および佐分利[30)] 原田ら[31)32)] 黒沢[33)] がある。

そもそも分類学において形態的形質を重視すべきは当然のことで，微生物といえども例外ではない。しかも最近のことにいちじるしく電子顕微鏡的観察が進んで来つつあるとき，とくにその微細構造すらも当然問題とすべき時期にきているといえよう。

(第1図) Pridham ら[3)] の Sporophore の分類

1. Straight		Section *Rectus-flexibilis*
2. Flexuous		
3. Fascicled		
4. Open-Loops Primitive Spirals, Hooks		Section *Retinaculum-apertum*
5. Open Spirals		Section *Spira*
6. Closed Spirals		
7. Monoverticillate		*Monoverticillus* Section
8. Monoverticillate with Spirals		Section *Monoverticillus-spira*
9. Biverticillate		Section *Biverticillus*
10. Biverticillate with Spirals		Section *Biverticillus-spira*

飯塚[34)35)] は，*Aspergillus* 属の胞子について電子顕微鏡（以下 EM と略）観察をおこない，充分分類の基礎となりうることを示した。阿部[36] は，*Penicillium* 属の分類にあたって同様な実験をおこない，EM 的特徴を認めている。

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放線菌胞子についても EM による観察はある程度おこなわれている。すなわち，Flaig ら[37] Enghusen[38] の観察などがあるが，いづれも明確な菌株名，あるいは菌株の性質の記載をかくもので，現行の分類と，EM 観察の結果がどの程度の関連性をもっているかほとんど知ることができない。Flaig らは約500株を用いて，第1表に示すように EM 的胞子形態を分類して[illegible]

(第1表) Flaig[37] らによる放線菌胞子の電子顕微鏡[illegible]

- Lang-oval
- rund
- glatt
- rauch
  - a) stachlig
  - b) harrig
  - c) warzig
- rechteckig

また，Baldacci および Grein[39] は，Baldacci の分類に従った種々の Series の *Actinomycetes* 約50株の胞子を EM 観察し，胞子の形態，電子線の透過性表面のようすをしらべ，これらの結果，各seriesの菌株間には胞子形態のある程度の共通性を認め，series 分類の一つの手掛りとはなるが，species の決定に用[illegible]

ところで，このように放線菌の分類に関しては問題が多いのであるが，Waksman あるいはKrassilnikov らも，現在では，まだ決定的な分類をおこなう段階ではなく，研究者のもつすべての知識を分類の key として[illegible]のような有様にかんがみ，著者らは EM 的微細構造が，従来用いられている分類形質とどのような関係があるかを知ろうと[illegible]，主として aerial myceliumの形態と，胞子の EM 的形態とを組み合わせて考察[illegible]果 2，3 の知見を得た。もちろん本実験は供試菌株の数も少く，形態的形質の遺伝的安定性を充分調べたわけでもないので，予備実験的段階にすぎないが，ここに報告する。

材料および方法

今回観察した192株中，64株は当研究所保存および長尾研究所より分与を受けた type culture であり，108 株は原田らにより土壌より分離された抗生物質産生株，その他，Baldacci より分与された25株中の21株（*S.* sp. No. 1-No.25）である。

培地は主として starch Bennet's agar および，Bennet's agar を用いたが，この他 soluble starch，potato ext., glucose soybean agar になどを用いた場合もある。いづれの菌株も 28° にて 2～3 週間培養し，充分胞子を形成させた後，膜を張ったメッシュを直接おしあてて EM 観察をおこなった

また，sporophore の形態は，agar plate 上で直接検鏡した。sporophore の分類は前述した Pridham [illegible]方法に従い，次の7つにわけた

1. *Rectus-flexibilis* (RF)
2. *Retinaculum-apertum* (RA)
3. *Spira* (S)
4. *Monoverticillus* (MV)
5. *Monoverticillus-spira* (MV-S)
6. *Biverticillus* (BIV)
7. *Biverticillus-spira* (BIV-S)

結　果

観察した193株中，大部分は RF (91株) RA (16株)，および S (83株) に属する。

1．胞子の形および大きさの斉一度について

[illegible]研究者[illegible]とほぼ同じであり，大別すれば次の3つになる。

(1) spherical or oval
(2) ellipsoidal
(3) cylindrical

このような形は，胞子の形成方式(たとえば，fragmentation とか segmentation とか) に当然関係することであろう 上記の3形の間も，連続的に変化すると考えてよく，同一菌株の胞子の中にも spherical と ellipsoidal が混在していたり，ellipsoidal にしても，その長径と短径の比率はかなり大きく変化するこ[illegible]かなり特徴的なものであるかもしれない。たとえば，*S. griseus* は7株ともかなり斉一な分布を示していた。*S. sahachiroi* 及び 238 B 1 にみられた柿の種形の胞子は特徴的である。又ツヅミ形 (⊂⊃) ともいうべき形の胞子は，*S. lavendulae* をはじめとして，ほとんどの菌株に多かれ少かれ認められたが，[illegible]は更に検討したい。

2．胞子の表面のようすについて

胞子の表面の構造もかなりいろいろなものがあり，観察した範囲では次のようである。

(1) smooth　(2) rough　(3) granular
(4) short-spiny　(5) spiny　(6) hairy

すでに報告された wrinkled は著者らの観察の範囲では認められなかった。

上記のような表面構造の変化も連続的と考えてよく，たとえば，*S. lavendulae* ATCC 8664 は rough であるが *S. rutgerensis* 2株にみられた short-spiny に極めて近いものといえるし，spiny にしても spineの長さに長短があり，その太さも異り，74B5株のごときは spinyであるが hairyに近いものである。また，*S.* sp. No.20においては，成熟に従って spineはやや長くなる。

表面構造中とくに明瞭な granular, short-spiny, spiny および hairy は第2表にみられるように，すべて spiral を形成する *Spira* に属することは注目に価する。他の報告者の写真よりみても，Baldacci および Grein の Series Diastaticus sp. Diastaticus および *Actinomyces* Cp. 936 はいづれも spineをもち，かつ *Spira* に属すると推定される。

著者らの観察した *Spira* 85株中，このような表面構造を示したものは20株であった。(85株中2株はBIV-S)。

(第2表) sporophore の形態　胞子の形，大きさの斉一度，および表面のようすによる分布。

Sporophore	Uniformity	Marking of Surface		Numbers of Strains		
				Type Culture	Newly Isolated	from Baldacci
R F 91	Uniform 77	Smooth	75	30	39	6
		Rough	2	0	2	0
	Irregular 14	Smooth	13	3	9	1
		Rough	1	1	0	0
R A 16	Uniform 11	Smooth	10	1	8	1
		Rough	1	0	1	0
	Irregular 5	Smooth	4	3	1	0
		Rough	1	0	1	0
S 83	Uniform 70	Smooth	51	14	28	9
		Rough	1	0	1	0
		Granular	1	0	0	1
		Short-Spiny	3	2	1	0
		Spiny	14	4* (3)	7	3
	Irregular 13	Smooth	10	3	7	0
		Rough	2	2	0	0
		Hairy	1	0	1	0
BIV 1	Uniform 1	Smooth	1	1	0	0
BIV-S 2	Uniform 2	Smooth	1	0	1	0
		Spiny	1	0	1	0

*S. sp. E-150 を含む。

考　　察

胞子形態は大別すれば spherical, ellipsoidal および cylindrical になるが，この間は連続的であり，ellipsoidal といっても長径と短径の比率の変化で spherical に近いものから long ellipsoidal までいろいろ変化し，同一菌株中さらに1本の spore chain 中[illegible]

（第3表）*S. griseus* 群の育成歴史

Strains	Metabolic Products	Cultural History
♯1	SM, V. B_{12}	Waksman's original
SN-14	SM-	Okami's red mutant
S-1	SM, V. B_{12}	Umezawa-Okami's original
SX_2-O-11	SM, V. B_{12}	S-1→Xray, 2 times→ monospore
H-12	Grisein	Okami's Grisein Producing strain
TS-601	SM, V. B_{12}	Sakai's original
MC-33	SM, -V. B_{12}	Z-38→mono spore

[illegible] irregular [illegible] 株の一つの特徴である場合がある。*S. lavendulae*, *S.* [illegible] かし，*S. griseus* は斉一の方の例であって第3表に示すように，[illegible] うな産生物質もちがうがかなり斉一な結果を示している。このことは形態的形質がかなり安定なものであることを示す一つの証さともいえよう。

このように，original の相異，X 線照射，産生物質の相異などがあるが，いづれも Bergey's manual に従って *S. griseus* と命名されたものは，その胞子の形態も，斉一度も，表面構造もほとんど等しく，この場合は安定した形態形質と生理学的性質が[illegible]したものといえよう。しかし，*S. fradiae* のように，3535株は RF に属し，117 株および岡見氏の原株は RFに属するような不一致，また，*S. olivaceus* 7 株の中smooth 6株 rough 1株のようにばらつくこともある。*S. olivaceus* では胞子形態，大いさの斉一度も又一定せず，7株中斉一なもの5株，不斉一なもの2株となっている。

原田ら[31]の研究によれば，sporophore の形態，aerial mycelium の色調，soluble pigment, carbon source の利用能，抗生物質の産生能などについて segregants におけるこれらの性質の変動を調べ，その結果，形態的形質は非常に安定したものであり，また，能美[28]によれば，spiral の形成のような形質は，生理学的性質（たとえば硝酸塩還元性，澱粉分解力，蛋白質分解力など）に比してはるかに安定な形質として遺伝することを示している。

このように，形態的形質は遺伝的にきわめて安定なものであれば，当然分類のkeyとして重視さるべきであろう。遺伝的安定性については，さらに検討を要する。

また，著者らの観察のごとく，spine およびその他の表面構造を形成する菌株の sporophore は，いづれも spiral を形成する *spira* に属する（*spira* 85株中20株において表面構造をみとめた）が，このことは無視し得ない結果と考えられる。しかし，spine 形成等の遺伝的安定性のごとき点については[illegible]な検討をおこなっていないので，この点を指摘するに止める。

また培地組成と胞子形態の関係は，当然菌株の栄養要求の問題として検討されるべきであるが，この点については次の機会におこないたい。

要　　約

193 株の放線菌の胞子につき電子顕微鏡観察をおこない，Pridham らの sporophore の分類に従った sporophore の形態と，胞子の電子顕微鏡的形態の連性をしらべ電子顕微鏡的微細構造を分類上利用しうる可能性を示した。

1．胞子の形は spherical, ellipsoidalおよびcylindrical に大別されるが，この間は連続的に変化するもので，同一菌株についてもかならずしもその形態，大いさの斉一度は一定のものではない。

2．胞子の表面構造については smooth, rough, granular, short-spiny, spiny および hairy にわ

けられる．著者らの観察した範囲では wrinkled は認められなかった．

3. granular, spiny および hairy のごとき構造をもつ20株は，いづれも *spira* に属していた。

謝　　辞

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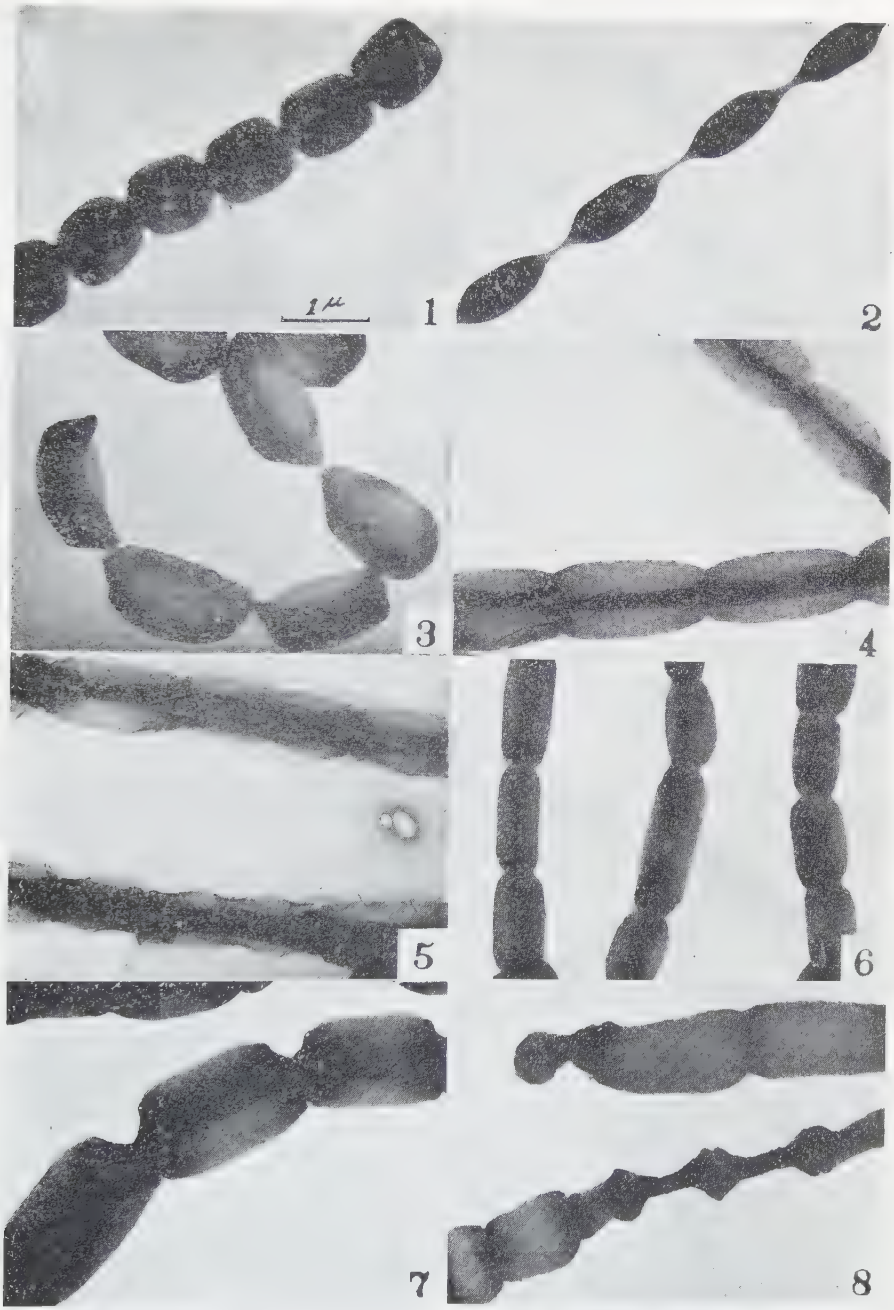
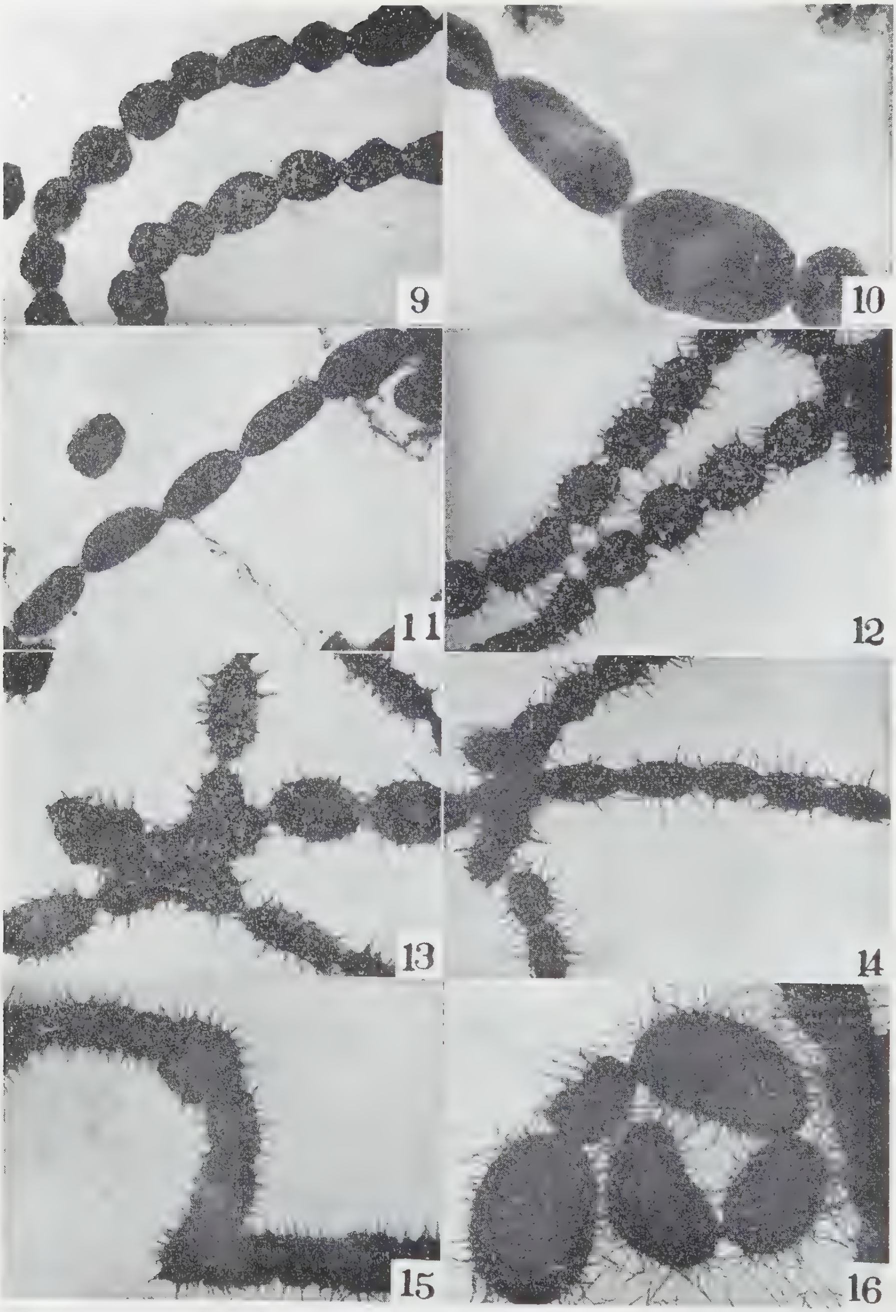
Summary

The spores of 193 strains of *Streptomyces* were observed with an electron microscope. There are some relationships between the morphology of sporophores classified by Pridham *et al* and the electron microscopic fine structure of the surface of the spores.

The morphological observation with the electron microscope may be available and feasible for the classification of *Streptomyces*.



1. *S. scabies* CBS (IFO 3111) 斉一な spherical ないし短い cylindrical を示す。
2. *S. rimosus* NRRL 2234 典型的な原形質連絡がみられる。fragmentation による胞子形成の代表的なものと考えてよいであろう。
3. *S. sachachiroi* 柿の種形をしている。電子線透過性。
4. *S. coelicolor* NI 9023 spore chain を貫いて1本の dense な，おそらく原形質と考えられる紐状構造がはしる。この細い紐状構造の他は電子線をよくとおす。
5. *S. tanashiensis* 289 A 1 *S. coelicolor* に近似しているが，原形質は太い。被膜に注意。一般にこの時期では被膜はほとんど autolysis をおこし，不明瞭なるがこの菌株ではきわめて鮮明である。
6. *S. griseus* #1 (Waksman) ellipsoidal ないし cyiindrical. *S. griseus* 群は，一般にこのような形と斉一性を示す
7. *S. flavochromogenes* H-3206 spore の連絡部位に注意。
8. *S. lavendulae* ATCC 8664 spore の斉一度はきわめて悪く，表面は細い小凹凸を多数示す。これらの事は本菌株の特徴である。



9. *S.* sp. No. 7 一面に多数の granular 構造がみられる。
10. *S. rutgersensis* H-1159 表面は微細な三角状の突起で被はれる。delicately spinulose とでもいうべきか。
11. *S.*sp.25B1-2 少数のごく細いspineがみられる。
12. *S. viridochromogenus* CBS (IFO-3113) 典型的な spiny 構造。spore の連絡部位に注意。
13. *S. purpurascens* NRRL-B-1454 Spine は太く，先端は鈍である。
14. *S.* sp. No. 19 spine は細く，長い。
15. *S.* sp. 74B5 spiny ではあるが，この spine は先端がきわめて細く，長く，かつ屈曲しており，hairy に近い。
16. *S.* sp. 172B6 典型的な hairy である。

1. Shapes of the spores were classified into three groups, namely, spherical, ellipsoidal and cylindrical. These three shapes change continuously. The shape and uniformity of the spores were not constant even in one spore chain.

2. Markings of surface of the spores were classified into such groups as smooth, rough, granular, short-spiny, spiny and hairy. But the present authors could not observe wrinkled spores.

3. It is a remarkable fact that all of the twenty strains which show clear marking of surface of the spores belong to the *spira*.

雑　　録

人工培地上の花粉の置床密度が発芽と花粉管の伸長におよぼす影響*

有　安　　勉**

Tsutomu ARIYASU**: Density Effect of Pollen Put on Artificial Culture Media, upon its Germination and Pollen Tube Growth.

1959年6月1日受付

[illegible]花粉管の伸長度を異にすることは，しばしば報告されている[1)2)]。木原[3)] (1919) は乾燥，低温で貯蔵した花粉を寒天培地にまいた場合，一般に密集した部分から花粉管を出すことが多く，ことに130日間貯蔵した *Digitalis purpurea* の花粉は密集した場合のみ発芽したという。Brink[4)] (1924) は *Cucumis sativus* および *Vinca major* の花粉を用い，寒天培地上では密集花粉の管伸長は，単離花粉のそれより良好であったことを実験的にたしかめた。Schmucker[5),6)] (1933, 1935)は *Nymphaea tetragona* および *Grevillea robusta* の花粉の液体培養で，あつまきにすると時として発芽が促進されることを見，Schoch-Bodmer[7)] (1936) は徐々に水分を吸収させて発芽させた場合，*Corylus heterophylla* の花粉が密集した部分で多数発芽したと報告している。さらに Kuhn[8)] (1938) は *Matthiola incana* の花粉がゼラチン培地上で，久保[9)] (1954) は *Cosmos bipinnatus* の花粉が寒天培地上で，いずれも密まきの部分でよく発芽したと報じている。以上の観察のうち，この事実を実験的に数値をあげてたしかめたものとしては，わずかに前記 Brink の報告があ[illegible]的吟味が必要と考えられるので，筆者は数種の花粉を用いて本現象を検討し，若干の結果を得たのでここに報告する。

材料と方法

[illegible]り扱いやすい，ツバキ(*Camellia japonica* L.)，チャ(*Camellia sinensis* L.)，キショウブ(*Iris pseudacorus* L.) ジャコウエンドウ (*Lathyrus odoratus* L.) およびオオマツヨイグサ(*Oenothera Lamarkiana* Ser. [illegible] すなわちショ糖10～15%含有の1～1.5% 寒天片（あ[illegible] 2mm.) (pH6.0～6.5)を用[illegible]場合は 3mm.×3mm.，花粉管長の測定には 10mm.×10mm.の[illegible]イドグラス上に3～10枚づつ並べて置き，各寒天板上に細毛*** で1粒宛拾った開葯当日の花粉を1～10粒

* 日本植物学会第19回大会 (1954) において一部を発表した

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*** よく洗つた1本の豚毛をガラス管の先端にパラフィンで固定したもの。

ように置床した。置床花粉粒間の距離は平均 100μ とし，各寒天板上の置床粒数に応じてそれぞれ1粒区，2粒区，……10粒区に区別した。発芽率測定の場合は5粒区まで，花粉管長測定の場合は10粒区までを設けた。この際2粒以上を置床する場合には花粉粒が互いに重なることなく接近させた。このようにしたプレパラートをペトリー皿におさめて室内に置き（室温約25°），一定時間後にプレパラートをとり出してアセトカーミンで固定して発芽率を測定し，オブジェクト・ミクロメーターで花粉管長を測定した。なお比較のため懸滴培養も行なった。この場合はVan Tieghem Cellと，10〜15%ショ糖液（pH6.0—6.5）を用い，懸滴の大きさ（直径約 5 mm.）をできるだけ等しくすることに留意した。

第1表 寒天培地上の花粉置床密度と発芽との関係
(数字は発芽率(%)，( )内数字は観察粒数，室温25°)

置床後時間	粒数	*Camellia sinensis*	*Camellia japonica*	*Iris pseudacorus*	*Oenothera Lamarkiana*
1時間	1	33.3(126)	32.9(429)	41.0(194)	4.3(232)
	2	60.0(478)	15.3(480)	51.0(108)	33.3(150)
	3	75.0(357)	55.5(324)	54.1(222)	47.5(42)
	4	78.6(420)	55.5(108)	59.0(84)	62.5(32)
	5	82.0(350)	74.0(150)	80.0(50)	76.6(60)
2時間	1	35.0(161)	43.6(472)	41.9(315)	35.9(492)
	2	62.3(392)	66.6(518)	58.1(444)	50.0(220)
	3	86.9(294)	72.0(414)	62.1(282)	59.0(240)
	4	92.5(364)	75.0(244)	64.5(200)	70.4(196)
	5	95.8(315)	82.2(245)	82.4(85)	84.4(180)

結　　果

I. 寒天培地

(1) 発芽に対する影響

チャ，ツバキ，キショウブ，オオマツヨイグサの花粉を用い，置床1時間および2時間後にそれぞれの発芽率を調べた。（第1表）

ここにあげた発芽率は各区の置床全粒数に対する発芽総数の百分率である。第1表から知られるように，供試4種の花粉はいずれも密集するほど発芽率がよくなっている。1粒区と2粒区との差異はいずれの場合も明らかである。すなわち1粒区では一般に発芽率は30〜40%前後にとどまるが，2粒区では50〜60%以上となっている*。とくにオオマツヨイグサの場合，置床後1時間では1粒区は 4.3%しか発芽しないが，2粒区では33.3%の発芽率を示した。1粒区ではオオマツヨイグサを除き，1時間後も2時間後も発芽率に大差がない。他の区では1時間後よりも2時間後の方が多少とも発芽率が上昇している。この場合も粒数の多い区ほど発芽率を増している。

(2) 花粉管の伸長に対する影響

チャ，ツバキ，キショウブおよびジャコウエンドウの花粉を用い，置床後2時間で固定し各区の花粉管長を測りその平均管長を第2表に示した。（第2表）結果は発芽の場合と同様に，供試4種花粉とも1粒区よりも多数粒区において明らかに伸長は良好であった。しかし粒数が多くなるにつれ，管伸長が目立つてよくなるのは一般に3〜6粒区まで，それ以上ではわずかに伸びるかまたはほとんど差がない。1粒区と2粒区における管伸長の差異が，発芽率と同様にもっとも著しく，ジャコウエンドウを除き，いずれも他の1粒の差をもつ区間での管長の差よりも大きい。

第2表 寒天培地上での花粉置床密度と花粉管伸長との関係
(置床後2時間後の結果，数字は管長(μ)平均，( )内数字は測定花粉粒数，室温25°)

粒数	*Camellia sinensis*	*Camellia japonica*	*Iris pseudacorus*	*Lathyrus odoratus*
1	170(209)	200(208)	260(172)	50(251)
2	300(165)	350(311)	460(215)	100(183)
3	340(231)	400(194)	500(203)	150(161)
4	350(362)	420(191)	540(263)	160(231)
5	360(107)	430(173)	550(127)	200(145)
6	380(144)	440(210)	600(200)	200(164)
7	380(179)	450(205)	610(195)	210(208)
8	390(194)	460(180)	620(106)	220(141)
9	390(214)	460(173)	620(142)	220(193)
10	400(149)	460(188)	630(228)	230(218)

* このような1粒区〜2粒区間の発芽率の差異は統計的にも有意の差が認められる

Ⅱ 懸滴培地

寒天培地上と同様な傾向が液体培地においても見られ[illegible]よって検討した。（第3表）

第3表 懸滴培養での花粉粒数と発芽および花粉管伸長との関係

材料 *Iris pseudacorus*（室温25°）

置床後時間	花粉粒数	観察花粉粒数	発芽率(%)	花粉管長(μ)
1時間	1	73	28.7	20
	2	98	45.9	80
	3	63	49.2	90
	4	92	50.0	85
	5	75	50.7	100
2時間	1	67	26.8	70
	2	90	46.7	160
	3	60	48.3	190
	4	84	67.9	240
	5	65	67.8	200

その結果は，寒天培地同様に1粒区よりも多数粒区において発芽および管伸長がよかった。しかし2粒以[illegible]であるという傾向は寒天培地上のように顕著ではなか[illegible]

考　　察

以上の結果からこの実験に供された花粉では人工培地に1粒づつ置かれた場合よりも多数集つている方が[illegible]る。本研究であつかった種類は4科にすぎないが，従来[illegible]と10数科におよぶ。しかもこれ等の科は類縁関係が近[illegible]象がみられるものと推察される。本実験に用いた植物の花粉では5〜6粒が密集して一群となるとき，寒天培地上で最大に近い花粉管の伸長を示す。これに反し発芽率の場合は6粒以上を1群としてもなお発芽率上[illegible]志佐[10]（1934）も述べているように，置床法，あるいは測定法にとくに注意する必[illegible]

このように人工培地に密にまかれた花粉の発芽や花粉管の伸長が，個々に単離しておかれた花粉のそれらより良好である原因については，花粉または花粉管から浸出する物質を想定する考え[4][8][11]と，吸水の制限[illegible][3][9]，今なお問題として[illegible]されているしかし本研究で行なったキショウブ花粉における寒天培養と懸滴培養との結果の比較から，花粉からのある種の物質に原因を求めうる可能性が見出される。すなわち，当然有効物質が稀釈されると考えられる懸滴培養では，密度効果は寒天培養に比し明かに劣る。しかし1粒区〜2粒区間の差はなお明瞭であるから吸水[illegible]見做されがたい。なお[illegible]の場合有効物質の存在を仮定するなら，その物質は，かなり水に拡散しにくいものと考えられるがこのことについて[illegible]

本研究は主として京都大学農学部応用植物学研究室で行なったものである。御指導を賜わった同学の今村駿一郎教授および東京教育大学理学部植物学教室の植田[illegible]助教授[illegible]謝意を表するとともに[illegible]京都大学農学部応用植物学研究室の渡辺光太郎氏に心から感謝の意を表明する。

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Summary

1. The present work was carried out to investigate experimentally if the germination percentage and the pollen tube growth are different among various densities of pollen grains put on sucrose agar media.

2. These pollen grains of the following plant species *Camellia sinensis*, *C. japonica*, *Iris pseudacorus*, *Lathyrus odoratus* and *Oenothera Lamarkiana* were used for this purpose.

3. The greater the number of the grouping grains became the more the germination percentage and the tube growth increased. The difference of the tube growth was most conspicuous when a single isolated grain was compared with the two grains grouping set side by side.

4. A group consisting of five or six pollen grains secures the best growth of the pollen tube on agar media, whereas more grouping grains are required to gain the best germination percentage.

5. It was discussed that certain substances might take part in the increment of germination percentage and the acceleration of pollen tube growth.

Short Communication

Osamu SHIBATA* : Photoperiodic Response of the Rice Plant as Affected by Iron-Deficiency

[illegible]

Received October 6, 1959

This paper represents an effect of short-day treatment on rice plant, variety Shinriki, cultured with different iron-levels resulting no visible difference in chlorophyll content. The plants were divided in two nutritional groups as shown in Table 1 on the 52nd day after the seed-germination. Fifteen treatments of short-day (8 hr. day-light) were experienced at the stage of five to six leaves, and the control plants were grown under natural day length.

Table 1. Photoperiodic responses of rice plant affected by iron levels in the culture solution. (May 1, sowed; July 19, short-day treatment started.)

culture solution	iron level	chlorophyll content*	days from sowing to heading		days accelerated
			control	short-day treatment	
	mg./l.		days	days	days
complete	1.129	0.48	145	120	25
iron-deficient	0.054	0.46	144	133	11

* per cent of chlorophyll to fresh-leaf weight.

Flower formation of etiolated plants caused by iron-deficiency has been reported to be significantly delayed or inhibited by some workers[1]. In these cases, it is supposed that the plants may produce the insufficient carbohydrates on account of reduced chlorophyll content. The data obtained here, however, revealed practically no difference in the chlorophyll content of the nutritional groups, though there was considerable difference in iron concentration of the culture solution. Their growth was also found to be almost normal to all appearance. Then, it was suggested that the difference in time required for the headings of the plants in the different nutritional groups might be caused only because of the different iron levels in culture solution. These facts may have some relations to the formation of the flowering substances or to certain processes following them. On the other hand, however, with a microscopic observation of the head-primordia, the writer failed to find any difference in time of the head-initiation. Therefore, it seems probable that the iron-deficiency affects the later stage of head differentiation and its further development.

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本　会　記　事

第 24 回（仙台）大会

9月4日（金）から6日（日）までの3日間，第24回大会は東北の地，仙台の東北大学川内分校で開催された。この分校は仙台市の西，旧日本陸軍の兵舎を改造したもので，市内を一目で見下すことのできる小高い丘の中腹にある。大会の前日の3日には，評議員会が開かれ，別項のような内容が報告，討議された。翌4日は午前9時より，A，B，C，Dの4会場にわかれて，一般講演がおこなわれ，つづいて午後3時からは，分類，形態，生態，生理のシンポジウムが開かれた。第2日目の5日も，前日とおなじく一般講演をもって始まり，午後からのシンポジウム，談話会等で夜おそくまで熱心な討論がつづけられた。最終日の6日は10時30分まで，一般講演をおわり，そのあと1時まで展示講演がおこなわれた。午後は3時から懇親会と総会とが続いて開かれたが，その前に新設の東北大学附属植物園の見学もおこなわれた。午後6時，総会の終了をもって，3日間にわたった24回大会はすべて終り，翌7日からは，松島，十和田，八甲田の見学旅行がおこなわれた。

評議員会　（9月3日午後6時，仙台ユネスコ会館）

出席者：評議員18名（欠席8名），会長代理，幹事長，幹事4名，大会準備委員1名

本田会長代理の挨拶につづいて，幹事長から，次の事項についての報告がおこなわれた。

1．役員移動。2．評議員改選。3．現在会員状況。4．33年度会計。5．34年度会計中間報告。6．35年度予算案。7．植物学雑誌刊行経過および予定。8．図書の交換，寄贈の状況。9．各種奨励金の推薦状況

とくに論議の中心となったのは，植物学雑誌の刊行についてで，雑誌のページ数を増して，論文の掲載をもっと早くしてもらいたい，という意見が述べられた。これに対して幹事長より，たゞ単にページを増すことは，郵送料の問題，会費値上げの問題もあり，簡単にはいかぬこと，さしあたりページ当りの行数を増すことによって，実質的な内容増加をはかりたいという答えがあり，各評議員もこれを了承した。

ついで近畿支部の小清水卓二評議員より，次の大会は大阪，阪大理学部・医学部で開催し，会期は11月1日から7日までのうち適当な日を選ぶこと，大会会長には三木茂氏，副会長には奥貫一男氏が選ばれている，との報告があった。なお36年度の大会は東京で開かれることになった。

総　　会　（9月6日（日）午後5時，東北大学川内分校大講堂）

本田正次会長代理の挨拶ののち，幹事長より次の諸事項の報告があり，総会出席者の承認を得た。

(1)　役員：移動の報告および承認（会長，評議員については本年4月号掲載）

(2)　現在会員の状況報告（昭和34年8月31日現在：1258名。うち名誉会員22名，特別会員22名，外国通信会員4名，終身会員54名，通常会員1156名）

(3)　会員移動の報告（昭和33年9月30日～昭和34年8月31日，新入会110名，再入会2名，死亡2名，退会14名，除名11名，差引増加85名）。

(4)　植物学雑誌刊行経過および予定の報告。（別表）

(5)　図書の交換，寄贈の状況報告（交換：国外受理90，国外発送80，国内受理33，国内発送32，寄贈：国外受理9，国外発送2，国内受理66，国内発送4，予約購読270）

(6)　昭和33年度決算の報告（昭和33年1月～昭和33年12月，本年3月号掲載）。

(7)　来年度大会に関する報告（前記，評議員会の項参照）

このあと，九州支部の山根銀五郎，近畿支部の吉良竜夫の両氏から，林野庁の拡大造林計画によって，原生林の破壊される恐れがあるが，植物学会はこれに対して何らかの手を打つべきではないか，との発言があり，総会の意志として本田会長代理が，文部省，学術会議に働きかけることが承認された。

植物学雑誌刊行状況

		論文数	ページ数
1956 年度	（小倉記念号）	97	602
1957 年度	（75周年）	77	438
1958 年度		63	448
1959 年度 1 月号		2	22

2 月号	5	38
3 月号	9	70
4 月号	8	50
5 月号	6	50
6 月号	5	44
7-8 月号	7	62
9 月号（予定）	5	44
10 月号（〃）	6	44
11-12 月号（〃）	9	86
	63	510

通　常　講　演

形態・分類・地理

西田雄行・斎藤真太郎：セン類の胞子発芽について（第2報） 1. イクビゴケ, 2. ハリミズゴケについて

加崎英男・岩崎尚彦：シャジクモ科植物の生長点の分化と器官形成 Ⅲ *Nitellopsis*

斎藤真太郎・下瀬敏：蘚類のさく歯の発生型式について

中沢信午：スギモクの仮根分化と粘質物の形態との関係

福田一郎：エンレイソウ及びその近縁種における花器[illegible]

[illegible]

上野実朗：花粉膜の微細構造（特にヤグルマギクについて）

粉川昭平：福井県下の中新統より発見された *Carya* の Calcified nut

小林艶子：ケイ藻 *Cymbella turgidula* var. *major* の変異について

奥野春雄：隠岐島の珪藻土について

熊野茂・広瀬弘幸・瀬戸良三：邦産緑藻 *Microthamnion* の生殖について

広瀬弘幸・熊野茂・瀬戸良三：邦産緑藻 *Chaetophora elegans* の発生についての二三の観察

福原[illegible]

新崎盛敏：ウシケノリの生活史について（第1報）

千原光雄：日本産カギノリ科植物の生活史, カギケノリ←→ファルケンベルギアの関係の新しい知見

長谷川由雄：[illegible]の生態学的研究（Ⅲ）生長と寿命とについて（追報）

加崎英男：日本産車軸藻類　第13報

[illegible]：[illegible]それらの胞子発生

瀬戸良三・熊野茂・広瀬弘幸：カワモズク属（*Batrachospermum*）の3種 Chantransia stage について

野田光蔵：満洲及朝鮮の藻類

野田光蔵・斎藤邦嘉：日本海の海藻(2)粟島，飛島附近[illegible]

山田幸男・中村義輝：噴火湾海藻の生態学的研究：潮間帯に於ける群落の遷移

香村真徳：沖縄島の海藻の分布

菅井道三：モエジマシダ原糸体の生長様式と炭素源

[illegible]：[illegible]ジャクの関係

[illegible]：[illegible]体に於ける分裂[illegible]の形成について

野津良知：ワラビの茎頂について

[illegible]

安藤久次：分布上興味ある日高南部の蘚苔類

真山三賀雄：放線菌 *Streptomyces* 属の分類に関する一考察

[illegible]：[illegible]新種（[illegible]第二次コロニーを作る菌株 No. 701）について

川戸峯子・信夫隆治：放線菌の一新種 *Streptomyces viridofaciens* について

増田染一郎：Myxobacteria の子実体について

[illegible]

小野幹雄：核型から見たカンアオイ類の系統

木村陽二郎：種の分化と supraspecies

林　俊郎：細胞の局部固定

加藤君雄：*Allomyces javanicus* var. *allomorphus* 類似[illegible]

[illegible]

椿　啓介：オニゲナ菌（ホネタケ）の純粋培養と子実[illegible]

細　胞・遺　伝

山崎典子：コアツモリ（*Cypripedium debile*）減数分裂の DNase 処理

平岡俊佑：核分裂にともなう核酸の消長　花粉粒の核分裂

石田政弘：*Zea Mays* の根端分裂組織の核酸量

深沢広祐：雄性不稔コムギ葯における核酸の消長

[illegible]

清水晃・藪野恭三：*Nitella* 節間細胞の切断とくくり

に関する研究

植田勝己：*Oedogonium* の細胞学的研究

八戸正夫：ユキノシタの葉の表皮細胞の原形質復帰時間について

吉田吉男：蘚および羊歯等数種の細胞に於ける硝酸銀還元反応の検討

森本　孝：ユーグレナの細胞分裂の阻害と呼吸阻害

三木寿子：他種雌ずいに対する花粉管の屈向性

加藤一男：柱頭反応の検出

伊倉伊三美：サトメシダ前葉体のルセニウム赤呈色反応，特に造精器裂開の二三の考察

湯浅明・大隅正子：精子形成とラセン糸

板垣史郎・古川稔・木下祝郎：放線菌胞子の電子顕微鏡による形態について

植田利喜造・和田優：葉の表皮細胞におけるプラスチッドの構造と発達

村上悟・植田利喜造：オオカナダモの葉細胞の微細構造

左貝アイ子：植物細胞のオスミウム固定についての電子顕微鏡的研究（第2報）

新家浪雄：高等植物の静止核の電子顕微鏡的構造

鷹取晟[illegible]：ソラマメの核分裂に及ぼす呼吸阻害剤の影響

馬場[illegible]吾：カルス形成の際に於ける細胞分裂とアスコルビン酸

佐藤七郎：細胞レベルと非細胞レベルでのＴＴＣ還元の比較

飯島　衛：前還元分裂と還元分裂における脱水素系の変化

中沢　潤：ムラサキツユクサ三倍体の花粉形成について

水野忠款：トキソウ（*Pogonia*）の体細胞分裂

重永道夫：核分裂における仁の行動について

佐々木正人：車軸藻類の細胞学的研究Ⅷ．造精糸の細胞分裂の同期性保存率と温度

高尾昭夫：二三のマメ科植物の胚発生について

近藤静代：ホウセンカの胚発生について

吉田　治：センリョウの胚のう形成

木村劼二：ウシグソヒトヨの二核性子実体（予報）

武丸恒雄：エノキタケにおける不和合性因子の遺伝子構成に関す定量的研究（１）

寺川博典　ヒラタケの不和合因子

稲荷山資生・竹村英一：ヒガンバナ属の人工雑種について（第２報）

中山　包：サボンソウの正逆雑種の偏母性とその年次的消長

竹中　要：染井吉野の起源

生理・生化

持塚洵・芦田譲治：銅耐性酵母の集団組成の変化Ⅱ

瀬野悍二・芦田譲治：酵母の銅馴養中における遺伝的変化

荒勝　豊：銅培地上における酵母の生長速度と生長様式の関係

菊池忠寿・芦田譲治：銅耐性酵母の硫酸還元能の遺伝

村山徹郎・芦田譲治：銅耐性酵母の有機酸及びアミノ酸代謝（Ⅱ）

中村運・芦田譲治：酵母のカドミウム耐性に於ける燐酸代謝について

内貴信夫：銅耐性酵母の硫化水素生成径路

高田英夫：酵母菌の表層核酸の生理的意義Ⅰ．浸透圧変化について

徳野真一・山本武・高田英夫：酵母菌の表層核酸の生理的意義Ⅱ．色素吸収と呼吸との関係

永井　進：色素類による酵母の呼吸欠損変異の誘起

柳島直彦：酵母の実験的 Dimorphism

三戸信人：酵母胞子形成に及ぼすアミノ酸の影響

野村一郎・三輪知雄：高等植物の硝酸還元酵素系に関する研究

大西智子・森健志：脱窒素反応におけるエネルギー代謝（Ⅰ）燐酸エステル化について

小野　林：*Azotobacter vinelandii* における有機酸代謝について

古谷庫造・黒田和子・三輪知雄：緑藻石灰藻類のリンゴ酸脱水素酵素作用について

中山弘美・井上行雄：*Streptomyces griseus* の物質代謝（Ⅷ）L-glutamic dehydrogenase

近藤武夫：マキの根粒について（第１報）

山田　保：葉粒菌と生長促進物質との関係

香山時彦：二三の淡水産藻類における呼吸と浸透圧の関係

田沢　仁：フラスモの浸透価に及ぼす外因の影響

田沢仁・永井怜子：フラスモの浸透調節におけるイオンの役割

森　祐二：単一植物細胞の浸透特性（第７報）

花房尚史：シャジクモの原形質流動に及ぼす界面活性剤の影響

上坪英治：原形質分離と原形質流動

秦野節司・中島宏通：フラスモ細胞のＡＴＰ含量とＡＴＰ分解酵素活性について

[illegible]：[illegible]について

賀来章輔：植物組織の凍結曲線の分析（２）

高見伸治：酵母の水分生理学的研究（Ⅱ）塩類濃度と[illegible]

大槻虎男・今井百里江子：好稠糸状菌の研究：*Aspergillus glaucus* var. *tonophilus* 以外の二菌株の好稠[illegible]

[illegible] 寛：*Penicillium restrictum* の生産する色素について

[illegible]：[illegible]について

高橋節也：チューリップの開花運動の反応部位の測定

[illegible]：[illegible]用物質の研究Ⅲ．子実体生長ホルモンの分離及び検定方法

小西通夫・萩本宏：担子菌子実体の生長を促進する作用物質の研究Ⅳ．ツクリタケ子実体の非酵素的ＩＡＡ[illegible]

小清水卓二・稲田悦子・泉節子・源ミツ・菅沼通子：蔓性植物の生長作用物質特に *Phaseolus* の場合

[illegible]

小林万寿男：茎の発根に関する一考察（第２報）

久世源太郎：側芽の伸長と抑制（第７報）

[illegible]：[illegible]キシンの作用

依田静子・芦田譲治：エンドウ黄化茎の可伸性と浸透[illegible]

橋本徹・八巻敏雄：光の葉生長促進作用の機作

橋本 徹：茎の肥大生長におけるＩＡＡとカイネチンの相互作用

勝見允行：黄化エンドウ茎切片に対するカイネチンの影響

藤原彰夫・小島邦彦：小麦分離培養根端における生長促進物質について

木下哲雄・柴田治：アサガオの粗蛋白水溶性区分の日長処理回数に伴う吸光度変化

奥田光郎：[illegible]処理をしたアサガオの内部形態的観察

滝本敦・池田勝彦：アサガオの日長感応に対する暗期後の光条件について

滝本敦・池田勝彦：アサガオの日長感応に対する自然薄明時の影響

[illegible]：[illegible]について

[illegible]：[illegible]を要する種子発芽の一[illegible]について

伊藤正・石川茂雄：種子発芽における physiological phase について

河原晨・高田英夫：ヒシモドキの発芽に関する二三の観察

石川重夫：種子発芽に対する金属キレート剤の作用

藤沢久雄・芦田譲治：大根子葉切除胚の吸水と呼吸

寺岡宏・宇佐美正一郎：コムギ胚の炭水化物代謝

[illegible]：[illegible]発芽時における[illegible]代謝

[illegible]：[illegible]

森 隆也：花粉の生理と炭水化物との関係について

沢田義康：*Lilium dauricum* Ker-Gawl（エゾスカシ[illegible]）の花粉の生理学的研究

三戸明・林克巳：水稲農林８号とその人為倍数体の生長にともなう組成アミノ酸の変化

林 克己：トウモロコシの芽生えにおいてアミノ酸の組成におよぼすＫイオンの効果

[illegible]：[illegible]の影響について（１）

安田 斉：アカカブ発芽種子に出現する authocyanin 対する dihydro-streptomycin の影響

[illegible]：[illegible]体内における P^{32}[illegible]

山本昌木：*Phytophithora infestans* 菌に対する感受体[illegible]

宮地重遠・大浜多美子：光合成に関与する炭酸還元物[illegible]

[illegible]：[illegible]に於ける光化学的燐酸転移反応（第６報）

[illegible]：[illegible] *Cyanidium* の[illegible]反応について

岩塚孝・森健志：硫黄細菌の CO_2- 固定

鈴木 恕：高等植物 α- ガラクトシダーゼの糖転移作用によるラフィノース異性体の生成

鈴木武夫・三輪知雄・福井作蔵：*Chaetomium globosum* A２培地中の β-1.3′ xylanase 及び β-1.3′ glucanase との関係について

村上 進：起源を異にするガラクタンの酵素による加水分解

菅原 淳：精製葉緑体の RNase について

尾辻　望：大腸菌の lysed-protoplast による DNA 合成

松下亀久：タバコモザイクウイルスの増殖に関する研究

生　　態

渋川繁光：函館山におけるエンレイソウの生態

松坂　聡：エンレイソウ属における種間交雑

倉内一二：イチョウ個体性・続

堀川芳雄：日本の高山帯

塚田松雄：洪積世の花粉分析的様相Ⅱ．氷河期堆積物

島田正雄：花粉分析の基礎問題

堀正一・伊藤市郎：八島ケ原湿原に於ける Desmids 群落の遷移について

鈴木静夫：震生湖における水棲菌類の季節的変化

奥富　清：中国地方西部の暖帯林植生

宝月欣二・澄川沃・坂本充：[illegible]藻類の異常増殖について

楠元　司：常緑広葉樹の分布限界附近に於ける光合成生産

佐伯敏郎：植物群落における光の分配と光合成

沼田　真：マダケ林の群落構造と遷移

田崎忠良：光条件不足下に於ける栽培クワの生育について

髙橋基生・渡辺圧美：春化現象と根系呼吸

展　示　講　演

生　理・生　化

衣川堅二郎：マツタケの研究　子実体形成の要因及び生長

加藤一男・渡辺光太郎：イネ科柱頭反応

田中　清：アカマツ花粉の生長抑制物質について

堀　武義：ソラマメ芽生えの生長素の消長及びＩＡＡ種子処理の生長素への後作用

倉石　晉：カイネチン類縁化合物の葉の生長促進作用について

江口　享：シュウカイドウの無性芽形成に及ぼすトランス桂皮酸の影響

江刺洋司：シュウカイドウの無性芽中の生長調節物質と休眠の関係

長尾昌之・三井英二・熊谷孝美：シュウカイドウの無性芽及び種子の発芽に及ぼすジベレリンの影響

猪狩盛夫：イネの根におけるインドール酢酸の生体内変化

和田俊司：イネ子葉鞘に含まれるインドール酢酸酸化酵素阻害物質について

柴田万年・石倉成行：チューリップ花のアントシアンについて（其の１）

鈴木[illegible]：黄花ルピナスの芽生の L-lysine 酸化酵素（アミン酸化酵素）の性質と機能について

鈴木　昇：ヒドラジンと窒素固定

宮本義男：蠟又はパラフィンを唯一炭素源とする微生物培養

倉石　衍：酵母のビチオン及びパントテン酸欠乏による Unbalanced growth

井口昌一郎・大信武志：酵母の接合

奥田慎一：野生酵母 *Hansenula saturnus* の胞子形成

千葉保胤・藤茂宏・薬師寺英次郎・高宮篤・服部明彦：葉緑体成分の抽出と精製（Ⅰ），（Ⅱ）

鳥山英雄：オジギソウの細胞生理学的研究（第11報）

柴岡孝雄：オジギソウの活動電位とインピーダンス変化

遠藤沖吉：オジギソウの光刺戟，いわゆるoff-effectの発現条件

小田健二：シヤジクモの刺戟部位の活動電位

寺田　保：フラスモの電子顕微鏡的研究（第２報）

西崎友一郎：単色光による緑葉の光電反応について

高沖　武：水分欠乏植物における二三の酵素活性について

松田稔・高沖武：水分傾斜環境における植物の炭水化物代謝

細胞・分類・形態・生態

酒井文三：紀伊半島・四国のアゼトウナの染色体

小野記彦・永井静江：アゼトウナの変異性

西岡泰三：ヌリネニガナとイソニガナの雑種

藤原悠紀雄：*Aster* 属植物の核型分析（第６報）

下斗米直昌・杉山一：*Gymnaster Savatieri* における倍数性

加藤幸雄：オオバナクンシラン（*Clivia miniata*）の体細胞に見られる異常分裂（第１報）

辰野誠次：蘚苔類の小型染色体

清水芳孝：8mm. 顕微鏡シネ装置による原形質流動の撮影（１）トチカガミの根毛の cyclosis の異常型とキレート化剤の金属特異性の検討

堀川芳雄・関太郎：*Brotherella herbacea* Sak. ナヨナヨカガミゴケについて

佐藤正己：イワタケの分布と生態
越智春美：カサゴケ科蘚類基物の pH について
[illegible]
[illegible]
[illegible]分野——周縁キメラによる斑入葉を材料として
[illegible]
及川公平：二三のユリ科植物の Hypostase について
福島　博：オングル島及びその附近の植物
依田恭二・小川房人：タイ国の植生と土壌
菅原亀悦・飯泉茂：シバ白せん病地の植群
吉岡邦二・相馬寛吉：赤井谷地湿原の植物群落学的研究
石塚和雄・相馬寛吉：北海道東部の砂洲の最近における[illegible]変化
矢野悟道：草原に於ける植物地下器官の生態学的考察
[illegible]
[illegible]の問題点について
戸塚績・佐伯敏郎：縞枯山森林における水分経済と物質生産
高橋基生：樹種の生態学的特性と根系呼吸（第2報）

シンポジアム

分　類　シダの分類体系

9月4日　15.00〜18.00

座長：小倉　謙

(1)　伊藤　洋：胞子体（その1）栄養器官[illegible]
(2)　田川基二：胞子体（その2）生殖器官[illegible]
(3)　百瀬静男：配偶体の立場から

A会場で開かれ，参加者約80名．

伊藤は今回新発表の自己の分類系（各群を円で表わ[illegible]でのおもな分類系を互に比較し，問題の多い群を指摘[illegible]毛と鱗片などについてやゝ詳しく触れ，最後に一例としてオシダ科のメシダ群とチャセンシダ群（二三の分類系ではチャセンシダ科としてオシダ科と対立）の区別において栄養器官の形質が重視されていることを述べた。

田川は自己新編の「シダ類分類系対照表」(Diels, Christensen, Ching, Copeland, Holttum の5大分類系の科，亜科などの関係が一目でわかるもの）を[illegible]して用い，生殖器官すなわち胞子のう，胞[illegible]たか，たとえば胞子のうでは環帯の発達程度，形[illegible]重視され，[illegible]表面生の問題，その発生における同時生，順次生，混生の別などが問題にされることを示し，さらに，集合胞子のう群や acrostichoid のものに及び，次に実例としてヘゴ科が次第に分割されて小さくなっていった経過を説明した。

百瀬は100属500種にのぼる配偶体の比較形態学的な自己の研究結果をもとにして，胞子の基本形態，その発芽様式，原糸体，仮根，付属物，造精器，造卵器の七つが重要な形質であることを述べ，そのうち最も[illegible]る数個の大きな型分けに系統的意味のあること，さらに他の形質をも用いて配偶体による自然分類系が立て[illegible]殖を行なうものが新生のシダ群のうちの特定の群に偏在することは注目すべきことで実証的な分類体系究明の手がかりになるであろうと結んだ。

話題提供が終って小倉座長は，かって Bower が考[illegible]原寛，木村陽二郎，猪野俊平，中沢信午らによって，系統図示の方法の問題，栄養器官と生殖器官のどちらが重要かの問題，同じく配偶体と胞子体との関係の問題，形態の進化の問題などについての質問や意見の開陳が活発に行なわれ，小倉座長もさきほどの Bower のに化石と染色体[illegible]，時間一ぱい討論が続いた。

形　　態　I

過剰染色体　　9月4日　15.00〜18.00

[illegible]芳賀　忞

(1)　芳賀忞・野田昭三・茅野博・中村孝[illegible]・三宮正信：過剰染色体の二三の問題
(2)　清水芳孝：ヘラオオバコの過剰染色体
(3)　須藤千春：トウキビの過剰染色体

まず，芳賀を中心とする九大研究グループの，スイバをはじめ，いろいろの高等植物およびショウリョウバッタについての過剰染色体の広範な研究を綜括して茅野が演者として報告した。野生群個体における過剰染色体をもつ個体の出現頻度が多様（10%以下から50

%まで）であることおよび過剰染色体と異常凝縮（異質染色質化）の関係が述べられ，基本染色体と形態上の相違（短小化，端部動原体，腕相同染色体，不分離現象）から，過剰染色体の起原およびその後の進化過程を示唆した。遺伝的効果については自然個体群における過剰染色体の保持機構としてノヒメユリの胚嚢母細胞における珠孔側への逆担分離をあげ，基本染色体との不対合，外部形質への不関与等の共通の特性から過剰染色体が独自の遺伝的性質をもっていることを示唆した。

続いて清水はヘラオオバコの過剰染色体の減数分裂中の行動および過剰染色体と形質変化の関係を報告した。ヘラオオバコの過剰染色体は典型的な異常凝縮，後期の neocentric な行動の原因として，異質染色質代謝の異常を示唆し，このことが外部形質に対しても作用して，早熟矮性化および雌性から雄性への転化を誘導することを日長効果と関係づけて述べた。また，このような異常性から，この過剰染色体は異物的存在であり，その起原は比較的新しいとした。

最後に須藤はトウキビの過剰染色体についての従来の研究を綜説し，形態・発現頻度と数・表現的および遺伝的効果・起原にわたって述べた。そして核内異質染色質の量がトウキビの発育に関係あるものとし，A染色体上のコブの数の少ない品種集団にB発現頻度が高いことなどを例に，真正染色質と異質染色質の間に一つの均衡が存在し，それがトウキビの品種集団の分化に一つの方向を与えているかも知れないと結んだ。

これより一般討論に入り，座長より異質染色質を中心に論議することを提案，前述の3演者のテーマについて質疑応答があった後，小野(記)，水野の異質染色質に対する見解が述べられ，異質染色質を定義づけ，ことにこれを真正染色質と明確に区別することの困難性が指摘され，結局いろいろの角度からさらに異質染色質本質を解明することが，過剰染色体を追求する上に重要であるという結論が得られた。

形　態 II　茎の生長点と葉の形式に関する諸問題

9 月 5 日　15.00～18.00

座長　井上隆吉

(1)　原　　襄：生長点と葉の分裂組織

(2)　塙　　順：生長点に対する手術の効果

(3)　相馬研吾：生長点に対するオーキシンの効果

各話題提供後の会に活発な質疑応答がかわされた。大要は次の通りである。

猪野俊平――（原氏へ）Urmeristem と primary meristem との区別はどうか？　原――Urmeristem と言う言葉は使いたくない。Urmeristem は active なものかどうかは疑わしい。

猪野――原は gene と茎の生長点の分化との関係をどう考えるか？　原――生長点ではたしかに規則的な形成がみられ，それは genetic なものである。しかし両者の関係はまだ全く不明である。

中沢信午――各植物の分化形成はそれぞれ種に個有のパターンをもっている。原の場合は細胞の分裂軸や分裂域が種に個有でなければならない。塙の場合は生長の方向が“場”によって規定されると言うが，その“場”そのものが種に個有なはずである。相馬の場合は生長素に対する感受性の部域的差異，及び時間的におこる感受性の自律的変化が種に個有である。これらの点を三氏はどう説明するか？　原――遺伝的個有性と言う概念による以上に今のところわからない。今後その方面に進んで行きたい。塙――自分もその点をこれから究明してゆきたいが，いまの所 gene を考える以外にない。相馬――それは将来の問題で，今ではただ現象を実験的に解析してみたにすぎない。自律性は確かに存在する。

木村陽二郎――自律性とは種に個有のものでなく，共通のものである。中沢の考えはちがうのではないか。中沢――自律性そのものは non-specific であるが，自律性のあれわれ方は specific であると考える。

笠原基知治――遺伝学の立場からは histogen theory を支持せざるを得ない。なぜ形態では混乱するのか。外層が層になっていて内部がごまかされると言う事もあろうが，シーズンによってもちがうものであり，又種の系統によっても大きなちがいがある。種と言う事を充分考えないと混乱するのではないかと考える。

生　理 I　発育と弱光

5 月 4 日　15.00～18.00

座長　八巻敏雄

(1)　石川茂雄：種子の発芽に際しての弱光反応

(2)　滝木　敦：弱光と日長反応

(3)　藤井良平：胚軸の生長に於ける赤色光の影響

石川は光，温度の条件を変えることにより多数の種子の発芽過程の解析を試みた結果これを 7 つの型に分類して詳しい説明を行った。そして発芽に光を必要とする種子又は光により発芽が促進される種子の中に従来知られている赤色光(R)，近赤外光（FR）による可逆的光反応の見られないもののあることが指摘され，

た。討論は感光色素系についても幾分行われたが，休眠その他発芽そのものの問題にそれた感があった。

続いて滝本は花成と弱光反応の問題をのべた。アサガオでは暗期前FRが花成を抑制しそれはRで消却されるが，Rでの暗期中断による花成抑制はFRで回復しないことが示された。又暗期の短い場合には，その前または後に弱光がそえられると開花が促進されることを述べ，これは暗期反応の初期及び後期段階は光に比較的安定で弱光下でもすすみ得るためと考えた。なお低温で蔗糖が与えられると連続光下で花成がおこることが示された。更にムシトリナデシコの花成にはFRが必要であり，これにRが追加されると更に促進されることなどから感光色素系の複雑さが示唆された。

最後に藤井はミトリササゲの胚軸の生長に対するRの影響の問題をのべた。この場合にもR，FRによる光可逆系反応が認められること，Rの作用は生理的令の進行を促すものであることを述べた後，この反応に与る色素系についての知見を得る目的で種々の2価金属塩前処理の伸長に対する影響をR光下と暗処で調べた。金属塩はその作用から3つの型に分れたが，その中で暗処では阻害を示すがRの下では促進作用を示す型のHgは感光色素系を特別に阻害するものと考え，色素は蛋白であろうと推論した。この方法論については活潑な質疑応答があった。

綜合討論では感光色素の問題が論じられたが時間が不足のようであった。なお光合成研究者からは光の波長の純度，実験方向などについての示唆があった。

生　理 II　ジベレリンとオーキシン

9月5日　15.00～18.00

座長　芦田譲治

(1)　村上　浩：植物の伸長とジベレリン

(2)　加藤次郎・芦田譲治：ジベレリンの生理作用ならびにオーキシンとの相違と相互作用

(3)　八巻敏雄：ジベレリン，オーキシン，カイネチンの相互作用

まず村上はジベレリンとオーキシンが種々の生理現象に異なって作用する場合が多いことを示し，両者は異なった範ちゅうに入れられるホルモンであるとした。ジベレリンとオーキシンとの相互作用を認めはするがオーキシンは伸長反応においては切片で効くのに対し，ジベレリンが完全な，特に緑色個体に効くこと及び広い濃度範囲で抑制作用が見られないことなどから，植物の正常な生長発育に主役を演ずるのはオーキシンでなくてジベレリンであろうと述べた。これに対してはかなりの反対意見が出された。

ついで芦田はジベレリンとオーキシンが異なる作用物質であることを示すいろいろの事実を説明した。まず種々のオーキシン試験法でジベレリンは作用がないことをのべた後，両者の相互作用を検討し，両者の伸長増加作用は付加的であって，オーキシン—生長曲線の山の位置はジベレリン添加により移動しないことを示した。更にオーキシンは種々の抗オーキシンに対し競争的であるがジベレリンはそうでないことから，植物体内における反応受容体が両者の間で違うと述べた。

最後の八巻はジベレリン，オーキシンに更にカイネチンを加えそれぞれの作用を説明した後，これらの中の2物質相互の間だけでなく，更に3物質間にも相互作用のあることを示した。そして3物質を用いた場合の生理，形態的変化は細胞，組織内におけるこれらの種のホルモンの間のバランスとして考えるべきであると論じた。更に将来見出されると考えられるホルモンを含めその間のバランスを問題にすることにより植物におけるホルモン現象が明らかにされうるだろうという見解をのべた。

生　理 III　光合成の機作

9月5日　15.00～18.00

座長　高宮　篤

(1)　宮地重遠：放射性同位元素を用いた光合成機作の研究——“前照射実験”を中心として

(2)　千葉保胤・菅原淳・山本繁・大内精子：Hill反応の reconstruction への一つの試み

(3)　藤茂　宏：緑色植物の光化学亜硝酸還元系

各話題提供に対し大要次のような討論が行われた。

(1)　R—物質（前照射によって生成した炭酸固定物質）について種々討論された。すなわち，DPNH，TPNH の量の問題，光にだけ関係した TPNH での実験の可能性など，また，R—物質に相当するものとしてリポイック酸は考えられないだろうかの質疑があった。最後に座長より，Calvin の schema の否定から進んでいるこの仕事の中のR—物質をみつけ出すことに力を借すようにとの希望があった。

(2)　主に vitamin K の問題が討論された。Hill 反応の低下するときに vitamin K が抜けて行くのではないかとの質問があった。これに対し，vitamin K は抽出液にもなく，これを加えても反応の活性度が上らないことから否定的であるとの答えがあった。

(3)　8-オキシキノリン，NH_2OH の NO_2^- 還元および Hill 反応阻害に関するデータについて疑問が

出され，これらについての討論がなされた。また NO_2-還元については Hill 反応以外の経路も考えてみるべきであるとの意見が提出された。

生　態Ⅰ　下等植物の生態（その1）

9月4日　15.00～17.40

座長　宝月欣二

(1)　斉藤　紀：落葉の微生物的分解

座長　吉良竜夫

(2)　坂本　充：物質生産にもとずく植物プランクトン群落の量的把握

まず斉藤は，菌類を中心としたブナ落葉の分解者に関して，野外における落葉の分解様式の観察・これに関与する菌群の分離・同定から出発し，分解様式を異にする菌群・細菌間の相互作用およびサクセッションについて，実験的条件下の解析の結果を報告した。一方坂本は，生産者たる植物性プランクトン群落について，実験的に求められた光合成・呼吸と環境との個体段階における関連性から出発して，数値計算の適用により，実験的なプランクトン群落の発達を解析し，よって野外の湖沼における同群落の構造・変化・発達を理解しようとする方法論に立ち，その成果をのべた。

以上に対する論議は，(i) 実験の結果と野外の現象との関連性の問題，および (ii) 分解者と生産者の両者のある微生物群落におけるサクセッションの解釈の問題の2点に集中された。

即ち，斉藤に対しては，分離用培地（信夫），帽菌の採集法・菌根菌の役割（印東），落葉層および腐植の堆積速度（今関）などをめぐって，提示された実験的解析の結果が，野外の過程のどの点を反映するかについて，検討する必要のあることが指摘された。また沼田は，分解者における自発的サクセッションの動因をただし，そのクライマックスがゼロであるとする，Garret の仮説に対する疑問を表明した。

坂本に対しては，沼田・吉良によって，数値計算の前提とされた諸条件と，湖沼のじっさいの富養化との関連性が論議された。

生　態Ⅱ　下等植物の生態（その2）

9月5日　15.00～18.00

座長　佐藤正巳

(1)　鈴木兵二：蘚苔類の分布と生態（ミズゴケ類を中心として）

座長　堀川芳雄

(2)　田川日出夫・細川隆英・小村　精：着生性こけ類，地衣類の生態　特に研究上の特殊性

鈴木は，日本全土にわたるミズゴケ類およびその伴生種の分布調査の結果から，ミズゴケ各種類の分布型・ミズゴケからみた湿原の植生の型をまとめて報告し，欧洲の同種植生に関する Schwickerath の分類単位との対比をこころみた。また，田川らは，英彦山ブナ林の着生植物群落を中心とした，植物社会学的および生理－生態学的な研究の成果をのべながら，その途上に遭遇した着生蘚苔・地衣類群落の特異性・問題点を指摘した。

これに対する討論は，主として社会学的観点から行われた。まず安藤などは，蘚苔・地衣類では個体性・生長と繁殖の区別の不明瞭な点に関連して，標本地域のとり方の問題を論議した。また，越智・佐藤正などは，全層群落の単位と対立するものとして，Epilia などの着生植物群落の単位を別に設定することは不合理であるとする見解を示し，堀川は，全国的ないし全世界的な比較の上に立って単位を設定することの必要性を強調した。

生理―生態学的には，被着生木の生活力と着生植物群落との関係（菅原），高等植物と比較した蘚苔類の水分代謝の特異性などについて，2，3の質疑・討論が行わはれた。

懇　親　会

9月6日，於東北大川内会館，出席者約130名。予定が遅れて午後3時，係りの簡単なあいさつがあり開会。会は立食によるビール，ジュース，オードヽ ブル等の至極あっさりしたもので，殆んど補助なしの"take and give" 方式であったが，このやり方なら何処でも無理なくやれるものと思われる。

総会開始予定時間（午後4時30分）を考慮し，更に幹事の指示もあり4時会を閉じた。その間出席各位の御協力によって適度の流動もあり，歓談もなごやかに且つ盛んに行なわれ，会として一応その目的を達し得たものと思われる。

松　島　見　学

9月7日朝，参加者56名は定刻8時30分，駅前より貸切りバスを駆って都会の喧噪を逃れ，初秋の仙台平野を一路塩釜へと向う，長閑な田園風景を車窓に眺めながら多賀城趾を遙か左に，9時10分バスをすてて桟橋より遊覧船へと乗り込む。早朝より懸念された天候もからりと晴れ海上は凪いで絶好の行楽日和，材木島，仁王島等々の絵画的な島姿に魅せられながらガイ

ド嬢の説明に耳を傾ける。一方日頃の腕自慢はこの絶景をカメラにおさめんものとシャッターを切り続ける。船は種ガキの養殖場を経て，天候に恵まれてすら訪れる船も少い特別コースの外洋，更に狭い水道を通って宮戸島に寄港，一同湾内四大観の一つ，大鷹森展望台に登って小憩低倍率の松島八百八島を眺望し，12時30分松島海岸に上陸する。記念撮影の後名物のウナ丼で昼食，午後は海岸の古蹟をガイドの案内で見学する。旧藩公の菩提寺国宝瑞厳寺の重厚な気品と悠然たる佇まい，亭々空を摩す老杉と洞穴が織りなす閑寂境，湾に臨み緑の松を戴く国宝五大堂等々。2時30分自由行動にうつり夫々この天然の妙を満喫する。

4時半再びバスで「奥の七曲り」を登って双観山展望台より松島湾の景観に名残りを惜みつつ帰路につく。5時，無事駅前帰着，解散する。

八甲田山・十和田湖見学旅行

9月7日，大会終了後夜行で青森口より入った人は八甲田山登山に，また東北大学八甲田山植物実験所見学へと参加する。涼しすぎるほどだった仙台に比べて，晴天にめぐまれた第1日は，むしろ汗ばみながらの採集に見学にと本州北端八甲田山の針葉樹林湿原を歩きまわる。夕刻近く蔦温泉に着いた人は，ブナ・トチノキ・カツラ・サワグルミなどの原生林に囲まれた沼廻りをして一泊する。又十和田湖へ直通の人々は奥入瀬を逆上り5時間のバスの旅を終って湖畔子ノ口に着く。或る人は遊覧船で，又他の人はそのままバスで静かな十和田湖を観賞して湖畔（休屋又は宇樽部）に宿をとる。翌朝は曇天，有志は始発のバスで湖畔宇樽部に集り，和船で御倉半島の湖岸採集に半日を過す。

午後より雨，9日最終日も一日雨。

今回の見学旅行は，団体としてではなしに準備側で予定した3日間を参加者各自の自由な行動にまかせ，単なる旅行だけではなく，研究にも利用しうる態勢をとったことが特徴であった。後半の雨にわざわいされて，折角の山登りや湖岸採集を変更された人が多かった。3日間で旅行参加者70名，八甲田登山者19名，蔦沼廻り19名，湖岸採集13名。